Polhillia on the brink: Taxonomy, ecophysiology and conservation assessment of a highly threatened Cape legume genus

by

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DECLARATION

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TABLE OF CONTENTS

LIST OF FIGURES

Chapter 2

Chapter 3

Chapter 4

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

ABSTRACT

Polhillia C.H.Stirt. is a small, highly threatened genus in the mega-diverse Fabaceae. It is poorly known, and thought to include 8 species restricted to Renosterveld remnants in the Greater Cape Floristic Region (GCFR). Most of the species delimitation has been based strictly on morphology, and the monophyly of the genus has not been tested using molecular phylogenetic analysis. This was the first aim of this study. Results proved *Polhillia* to be monophyletic, with *P. involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte resolving as sister to the rest of the genus. Based on this, and unique pink or white flowers and dehiscent fruit (*versus* yellow flowers and indehiscent fruit), this species was placed into a new monotypic subgenus *Roseopolhillia* B.du Preez.

Given the confusing species delimitation, limited fieldwork history and weak herbarium specimen records of *Polhillia*, our next aim was to conduct a multidisciplinary systematic revision of the genus. This led to the recognition of 11 species in the genus, four of which are species new to science, while *P. canescens* was synonymised under *P. connata*. Conservation assessments based on IUCN criteria were conducted for all species. Two Critically Endangered species could be downgraded to Endangered based on the extensive fieldwork.

Polhillia species are distributed through Renosterveld fragments of the GCFR, with six of eleven species restricted to the Overberg region. Species, however, never occur in sympatry. The third aim of the study was thus to determine if restricted distributions of *Polhillia* species may be due to edaphic factors such as preference to certain topographic habitat requirements, soils or to rhizobial symbionts. Habitat observations proved to be crucial as species with overlapping distributions occupied substantially different topographic habitats. Closely related allopatric species in the Overberg also often occupied distinct habitats. Soil chemical analysis showed that Overberg species shared fairly similar soils, while non-Overberg species occur on substantially different soils. A shift in soil and habitat preference was found to be an important contributing factor in the evolution of *P. obsoleta* (Harv.) B.-E.van Wyk and *P. groenewaldii* B.du Preez. Rhizobial symbionts were successfully isolated from nodules of eight *Polhillia* species. Five species were nodulating with *Mesorhizobium* representing three distinct lineages, and three species with a single *Rhizobium* lineage. These results show an extent rhizobial symbiont preference, but closely related species often shared symbionts. This also represents the first finding of *Rhizobium* nodulating with a member of the Genisteae tribe.

OPSOMMING

Polhillia C.H.Stirt. is ‘n klein, hoogs bedreigde genus in die mega-diverse Fabaceae. Dit is swak bekend, en daar word tans geglo dat dit 8 spesies insluit wat tot Renosterveld fragmente in die Groter Kaapse Floristiese Streek (GKFS). Meeste van die spesie afbakening is streng slegs op morfologie gebaseer, en die monofilie van die genus is nog nie deur molekulêr-filogenetiese analise getoets nie. Dit was die eerste doelwit van hierdie studie. Resultate het bewys dat *Polhillia* wel monofileties is, met *P. involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte wat as suster tot die res van die genus uitkom. Gebaseer hierop, en die unieke pienk of wit blomme en oopspringende vrugte (*versus* geel blomme en nie-oopspringende vrugte), is hierdie spesies in ‘n nuwe monotipiese subgenus *Roseopolhillia* B.du Preez geplaas.

Gegewe die verwarrende spesie-afbakening, beperkte veldwerk geskiedenis en swak herbarium eksemplaar rekord van *Polhillia*, was ons volgende doelwit om ’n multidissiplinêre sistematiese hersiening van die genus te onderneem. Dit het gelei tot die herkenning van 11 spesies in die genus, vier waarvan nuut is tot die wetenskap, terwyl *P. canescens* onder *P. connata* in sinonimie geplaas is. Bewaringsassessering gebaseer op IUCN kriteria is vir alle spesies onderneem. Twee Krities Bedreigde spesies kon afgeskaap word na Bedreig gebaseer op uitgebreide veldwerk.

Polhillia is versprei oor Renosterveld fragmente van die GKFS, met ses van die elf spesies beperk tot die Overberg omgewing. Spesies kom egter nooit simpatries voor nie. Die derde doelwit van die was dus om te bepaal of die beperkte verspreidings van *Polhillia* spesies toegeskryf kan word aan edafiese faktore soos ’n voorkeur vir spesifieke topografiese habitatvereistes, gronde of rhizobium simbionte. Habitat waarnemings was baie belangrik, aangesien spesies met oorvleuelende verspreidings totaal verskillende topografiese habitatte bewoon. Naverwante allopatriese spesies in die Overberg bewoon ook dikwels kenmerkende habitatte. Die chemiese analise van grond het aangedui dat Overberg spesies relatief eenderse gronde deel, terwyl nie-Overberg spesies op substansieel verskillende gronde voorkom.

’n Skuif in grond en habitat voorkeur is as belangrike bydraende faktore in die evolusie van *P. obsoleta* (Harv.) B.-E.van Wyk en *P. groenewaldii* B.du Preez geïdentifiseer. Rhizobium simbionte is suksesvol geïsoleer uit die nodule van agt *Polhillia* spesies geïsoleer. Vyf spesies noduleer met drie aparte ontwikkelingslyne van *Mesorhizobium*, en drie spesies met ’n enkele *Rhizobium* ontwikkelingslyn. Hierdie resultate dui op ’n mate van risobium simbiont

voorkeur, maar naverwante spesies deel dikwels dieselfde simbionte. Dit verteenwoordig ook die eerste keer dat *Rhizobium* uit die nodule van ’n lid van die tribus Genisteae geïsoleer is.

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DEDICATIONS

I wish to dedicate this thesis to my long-time mentor and creator of the genus *Polhillia*; Prof. Charles H. Stirton who has played a pivotal role in my development as a botanist, taxonomist and legume geek. Having first come into contact with Prof. Stirton as a 20 year old boy on iSpot Southern Africa just starting to photograph a few pretty legumes seen on hikes, he inspired me to study legumes. Since discovering my first new *Aspalathus* species in 2013, he has taught me so much and pushed me to continue learning. It has been a humbling experience to learn about Cape legumes under his guidance over the past five years, and we are now collaborating on several Cape legume systematics papers.

I also wish to dedicate this thesis to all my friends and colleagues in the CREW program. I joined CREW in 2012 as a first-year student with very little plant knowledge and have grown so much since then. Various CREW members first pitched the idea of taxonomy as a career option, which I at first vehemently rejected. Mention must be made of individuals such as Ismail Ebrahim, Rupert Koopman, Dr Domatilla Raimondo, Lize von Staden, Dewidine van der Colff, Dr Tony Rebelo and Gigi Laidler for providing a platform for my growth within CREW and for pushing me towards a career in taxonomy. I would specially like to dedicate this thesis to the members of the Outramps CREW group in the southern Cape: Di Turner, Bill Turner, Jenny Potgieter, Marge Webster, Nicky van Berkel, Ann Symons, Gail Nootenboom, Rusell Bantich, Evie Bowen, Jean Purcell, Dave Underwood, Sally Adam, Sandra Falanga, Priscilla Burgoyne, Wendy Paisley and Mike Cameron. These individuals have been an incredible support to me since I joined the group in 2012, teaching me about plants and helping to grow my passion. I have great appreciation and admiration for all Outramps CREW members and will be forever grateful for the role they have played in my life. Finally, I would also like to dedicate this thesis to some CREW veterans who we have recently lost and who are sorely missed:

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CHAPTER 1

GENERAL INTRODUCTION

1. GREATER CAPE FLORISTIC REGION

The Greater Cape Floristic Region (GCFR) is a small region of extremely high plant diversity in the southwestern corner of the African continent (Linder, 2003; Manning and Goldblatt, 2012; Snijman, 2013). The GCFR covers an area of roughly 190 000 km^2 and is split into two sub-regions, namely the Core Cape Subregion (CCR; Manning and Goldblatt, 2012) and the Extra Cape Subregion (ECR; (Snijman, 2013). The floral diversity of the GCFR comprises of over 11 423 species of vascular plants, of which about 78% are endemic (Manning and Goldblatt, 2012; Snijman, 2013). These numbers are bound to increase over time as previously unexplored areas are explored and taxonomic outputs by botanists are increased. Generally, this incredible diversity may be attributed to diverse landscapes across the region with varying geology, topography and rainfall patterns (Linder, 2003; Manning and Goldblatt, 2012; Snijman, 2013). Additionally, the availability of micro-niches across the landscape combined with low levels of gene flow and dispersal have allowed species to evolve in small, isolated patches resulting in high levels of diversity (Kozak and Wiens, 2006; Linder, 2003; Wiens and Graham, 2005).

Two biomes dominate the GCFR, the Succulent Karoo and the fire driven Fynbos Biome which includes Fynbos, Fynbos Thicket, Western Strandveld and Renosterveld vegetation types (Allsopp *et al*., 2014; Manning and Goldblatt, 2012; Rebelo *et al*., 2006). The fire driven Fynbos and Renosterveld habitats occur mostly within the CCR, with some outlying inselbergs stretching as far north as Namaqualand (Manning and Goldblatt, 2012; Rebelo *et al*., 2006; Snijman, 2013). Renosterveld is a vegetation type associated with the presence and often dominance of *Dicerothamnus rhinocerotis* (L.f.) Koekemoer. and typically, only occurs on shale derived clay soils (Cowling *et al*., 1986; Levyns, 1956; Manning and Goldblatt, 2012; Rebelo *et al*., 2006). Although Renosterveld is typically dominated by Asteraceae and geophytic components (Allsopp *et al*., 2014; Cowling *et al*., 1986; Moll *et al*., 1984; Rebelo

et al., 2006), Fabaceae in many areas can form a large component of botanical diversity, particularly in recently burnt vegetation (Curtis *et al*., 2013; Dahlgren, 1988; Manning and Goldblatt, 2012).

The GCFR experiences a Mediterranean climate and falls predominantly within the winter rainfall region of southern Africa, although year-round rainfall is experienced towards the eastern edge of the region (Allsopp *et al*., 2014; Manning and Goldblatt, 2012; Rebelo *et al*., 2006; Snijman, 2013). Rainfall across the region is, however, extremely variable, ranging from 11 mm per year in Luderitz along the south coast of Namibia to over 2500 mm per year in Jonkershoek in the SW Cape (Manning and Goldblatt, 2012; Snijman, 2013). Rainfall is largely also dependent on the topography of the landscape as most rainfall systems move through from the south-west, leaving north-facing slopes drier than south-facing slopes (Manning and Goldblatt, 2012; Snijman, 2013). Tall mountains also create rain shadows that result in mountains being dominated by fynbos and lowlands often being home to Renosterveld or Succulent Karoo (Allsopp *et al*., 2014; Manning and Goldblatt, 2012; Rebelo *et al*., 2006; Snijman, 2013). Inland escarpments such as the Roggeveld are also dominated by Renosterveld growing at higher altitudes where rainfall and soils are not suitable for fynbos (Clark *et al*., 2011; Snijman, 2013; van der Merwe and van Rooyen, 2011).

Renosterveld across the GCFR has been largely transformed for agriculture due to the relatively nutrient rich soils available mostly in lowland habitats that are easy to farm on (Cowling *et al*., 1986; Curtis *et al*., 2013; Kemper *et al*., 1999, 2000; Rebelo *et al*., 2006). This habitat transformation has left many species threatened by extinction, with some already being extinct (Cowling *et al*., 1986; Curtis *et al*., 2013; Raimondo *et al*., 2009). Two of South Africa's most threatened plant genera, *Marasmodes* DC. and *Polhillia* C.H.Stirt. occur exclusively in Renosterveld and most species in these genera are on the brink of extinction due to habitat transformation for agriculture (Magee *et al*., 2017; Manning and Goldblatt, 2012; Raimondo *et al*., 2009).

2. LEGUME SYSTEMATICS

Fabaceae, commonly referred to as legumes, is a large family of angiosperms with a near global distribution and very high species diversity (Lewis *et al*., 2005; LPWG, 2013, 2017). The family is characterized by their pod fruit type (Lewis *et al*., 2005; LPWG, 2013, 2017; Tucker, 2003). The Fabaceae has the third highest species diversity within the angiosperms

globally, with roughly 770 genera and over 19 500 species currently described (LPWG, 2013, 2017). Legumes have been further divided into three subfamilies based mainly on morphology, namely the Mimosoideae, Caesalpinioideae and Papilionoideae (Lewis *et al*., 2005; LPWG, 2013; Tucker, 2003). Recently the Caesalpinioideae was found to be polyphyletic and a new classification of legumes was proposed whereby Mimosoideae was sunk into Caesalpinioideae, and four new subfamilies were described (LPWG, 2017). For the purposes of this study we will refer to the old legume classification, based primarily on the general morphological differences (LPWG, 2013; Tucker, 2003). The subfamily Mimosoideae is characterized by flowers that are densely aggregated into a racemose or paniculate inflorescence with radial symmetry and flowers that appear unspecialized (Tucker, 2003). Unique to this subfamily is the synchronous flowering of flowers on an inflorescence and undifferentiated organ whorls (Tucker, 2003). Caesalpinioideae is characterized by plants with very variable floral morphologies, with variable axes of symmetry, and with many examples of organ number reduction or loss (Tucker, 2003). The subfamily Papilionoideae is characterized by the typical papilionoid flower in almost all species (Cardoso *et al*., 2013; Lewis *et al*., 2005), that consists of differentiated flower petals consisting of one standard, two wing, and two keel petals (Lewis *et al*., 2005; Tucker, 2003). Globally this subfamily is represented by over 13 800 species and more than 478 genera (Cardoso *et al*., 2013; Lewis *et al*., 2005). Subfamilies are further divided into tribes based on shared unique morphological characteristics among several genera and phylogenetic relationships (Cardoso *et al*., 2013; Käss and Wink, 1997; Lewis *et al*., 2005). At the time of Tucker (2003), 30 tribes were recognized in the Papilionoideae, but Cardoso *et al.* (2013) reduced this number to 28 tribes. Only two tribes of Papilionoideae lack the typical papilionoid flowers, while the other 26 tribes have zygomorphic flowers with a descending cochleate pattern of petal overlap, which distinguishes them from the other subfamilies (Tucker, 2003). Papilionoideae is the most species diverse of the three subfamilies globally and within South Africa (Cardoso *et al*., 2013; Trytsman *et al*., 2011; Tucker, 2003).

A total of 24 legume tribes have been recorded within South Africa, 17 of which belong to the subfamily Papilionoideae (Trytsman *et al*., 2011). The most diverse legume tribe in South Africa is the Crotalarieae, which includes large genera such as *Aspalathus* L. and *Crotalaria* L. (Dahlgren, 1988; Trytsman *et al*., 2011). The Genisteae is a far smaller tribe both in terms of genera and species diversity, but shares many similarities in morphology and distribution with Crotalarieae (Lewis *et al*., 2005; Manning and Goldblatt, 2012; Tucker, 2003). Due to

many morphological similarities, the genera *Polhillia*, *Dichilus* DC. and *Melolobium* Eckl. & Zeyh., now phylogenetically shown to belong to the Genisteae, were previously placed in the Crotalarieae (Boatwright *et al*., 2008; Lewis *et al*., 2005; Polhill, 1976; Schutte and van Wyk, 1988; van Wyk and Schutte, 1989). The most evident morphological similarity between these genera and *Argyrolobium* Eckl. & Zeyh., which has always resolved within the Genisteae, is the fusion of the lower three calyx teeth to form a trifid lower lip (to variable extents at least) (Lewis *et al*., 2005; van Wyk and Schutte, 1989). The tribe Genisteae has been found to represent a strongly supported monophyletic clade comprising of at least 620 species in 27 genera (Kajita *et al*., 2001; Käss and Wink, 1997; Lewis *et al*., 2005). The genus *Lupinus* L. is globally the most diverse member of the tribe, while *Argyrolobium* is the most diverse genus in South Africa with 47 species recognized (Aïnouche *et al*., 2004; Edwards, 1994, 2005; Trytsman *et al*., 2011).

Polhillia is a small, fairly recently described, genus in the tribe Genisteae (Stirton, 1986a). It was described based on unique morphological characters such as connate stipules, which adnate to the petiole and sheath the stem, along with the presence of pseudo-peduncles, much reduced or absent bracts and bracteoles, and an unique combination of alkaloids (Stirton, 1986a; van Wyk *et al*., 1988; van Wyk and Schutte, 1989). Several included species were previously placed into other genera such as *Lebeckia*, *Melolobium* and *Argyrolobium* (Stirton, 1986a; van Wyk, 1992; van Wyk and Schutte, 1989), while *Polhillia ignota* Boatwr. remained undescribed, and was only recently formally described (Boatwright, 2010). *Polhillia involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte was originally described as *Argyrolobium involucratum* (Thumb.) Harv., then moved to *Melolobium* by Stirton (1986b), and finally included in *Polhillia* (van Wyk and Schutte, 1989). The genus is endemic to the GCFR, with five of the eight recognized species confined to lowland Renosterveld of the Overberg region, and three outlier species occurring in the Worcester, Swartland and Roggeveld regions (Curtis *et al*., 2013; Manning and Goldblatt, 2012; Raimondo *et al*., 2009; Snijman, 2013; Stirton, 1986a). One of the Overberg species was recently described (Curtis *et al*., 2013) and there is a putative new taxon, perhaps a subspecies, identified among material recently collected from the Suurbraak area (Stirton, 2017, *pers. comm*.).

This genus is in need of a complete taxonomic revision due to the presence of some unclear species boundaries. No full genus revision of *Polhillia* has ever been done, because the different species were incorrectly placed into various genera before the description of the genus by Stirton (1986a), and subsequent authors may not have used similar characters in

delimiting species. Morphology has been the most important suite of traits used in the description of new species and for taxonomic revisions, but new technologies and techniques allow for more accurate and in-depth studies. Macro-morphology remains an important part of taxonomic revisions and will be used in this study in conjunction with DNA sequence phylogenetic analysis, palynology and anatomical studies. Along with the revision, the validity of the genus *Polhillia* requires formal confirmation following the circumscription of the genus and subsequent studies (Stirton, 1986a, 1986b; van Wyk, 1992; van Wyk and Schutte, 1989).

Floral morphology is normally of considerable taxonomic value in angiosperms (Ornduff, 1968), although in the case of *Polhillia* and most other Papilionoid legumes, variation between closely related species is often very limited (Aïnouche *et al*., 2004; Bello *et al*., 2017; Dahlgren, 1988; Stirton, 1986a). Similarly flower colour is often systematically important (Johnson, 1996; Jones and Luchsinger, 1987; Rebelo and Siegfried, 1985), but legume genera rarely have much interspecific colour variation (Bello *et al*., 2017; Dahlgren, 1988; Edwards, 1994; Schutte-Vlok and van Wyk, 2011). Seven of the eight *Polhillia* species recognized, have yellow flowers and only *P. involucrata* has pink flowers (Curtis *et al*., 2013; Manning and Goldblatt, 2012; Snijman, 2013; Stirton, 1986a; van der Merwe, 2010). Petal size and shape is, however, an important measurement and is usually taken as an average measurement to allow for intraspecific variation (Bello *et al*., 2017; Boatwright, 2010; Dahlgren, 1988; Edwards, 1994; Jones and Luchsinger, 1987; Stirton, 1986a; Stirton and Muasya, 2016). Density and location of petal pubescence are also important to differentiate between species (Boatwright, 2010; Dahlgren, 1988; Edwards, 1994; Stirton, 1986a). The calyx is often more useful for species differentiation in many Papilionoid legumes than the corolla (Bello *et al*., 2017; Dahlgren, 1988; Kies, 1951; Moteetee and van Wyk, 2006; Schutte-Vlok and van Wyk, 2011). The length and shape of the calyx, along with the calyx teeth, are very useful for distinguishing between species, along with the absence or presence and density of pubescence (Bello *et al*., 2017; Dahlgren, 1988; Kies, 1951; Moteetee and van Wyk, 2006; Schutte-Vlok and van Wyk, 2011). Pseudo-peduncles are an extension of the terminal internode, which holds flowers and are uncommon in GCFR legumes, and are found mostly in *Polhillia* and a few *Argyrolobium* species (Boatwright, 2010; Edwards, 1994; Stirton, 1986a). The length of and number of flowers per pseudo-peduncle is important as some species have very long pseudo-peduncles, while others are very short (Boatwright, 2010; Edwards, 1994; Stirton, 1986a). Leaf morphology is widely used in plant systematics

as leaves hold many potentially informative characters (Jones and Luchsinger, 1987). Legumes are no different, as leaf morphology is often used for species discrimination among GCFR legumes (Bello *et al*., 2017; Dahlgren, 1988; Dludlu *et al*., 2015; Edwards, 1994; Kies, 1951; Moteetee and van Wyk, 2006; Schutte-Vlok and van Wyk, 2011). Although all *Polhillia* species have conduplicate leaves, leaf size, shape, colour and pubescence are helpful to distinguish between species (Boatwright, 2010; Stirton, 1986a). The shape, size and extent of connation of the stipules to one another and sheathing of the petiole are useful for identification purposes (Stirton, 1986a). The pod is often an important taxonomic character (Jones and Luchsinger, 1987; Lewis *et al*., 2005), also among several GCFR legume genera (Campbell and van Wyk, 2001; Dahlgren, 1988; Edwards, 1994; Moteetee and van Wyk, 2006). All *Polhillia* species, with the exception of *P. involucrata*, have indehiscent pods that are either laterally compressed or plicate (Stirton, 1986a; van Wyk and Schutte, 1989). Seed morphology of many of the species is unknown, but interspecific variation was found among the few species for which seed was available to Stirton (1986a) at the time of description. Although morphology is useful for separating species with clear species boundaries, phylogenetic studies will enable the identification of possible cryptic species, or show that some species have been split too finely, in addition to reconstructing the phylogenetic relationships between species.

Some *Polhillia* species have been included in wide-scale phylogenetic studies (Boatwright *et al*., 2008), but no study has focused on the genus and as such the monophyly is yet to be determined. The inclusion of the morphologically odd species, *Polhillia involucrata* into the genus by van Wyk and Schutte (1989) came after Stirton (1986b) circumscribed the species in the genus *Melolobium* rather than in *Polhillia*. We are now able to test the monophyly of the genus using molecular phylogenetic analysis and determine where this odd species belongs. It is important to determine whether the classification of *Polhillia* is too broad and if it perhaps requires splitting into subgenera or even describing a new monotypic genus. The phylogenetic relationships between the various species would be interesting to investigate, as this could help to identify morphological synapomorphies for the genus and also link to the disjunctive species distribution of the genus. Phylogenetic analysis will also enable to study the relationship between *Polhillia* and other genera in which species were placed before the description of the genus by Stirton (1986a), namely *Argyrolobium*, *Melolobium* and *Lebeckia*. Except for *Lebeckia* (Crotalarieae) all of these genera belong to the tribe Genisteae.

Palynology is widely used in systematic studies (Banks and Rundall, 2016; Dreyer, 1996; Zhao *et al*., 2016). Interspecific pollen morphological variation may be low due to strong selection against change in pollen morphology (Dehgan and Dehgan, 1988; Muller, 1979; Solomon, 1983), but many studies have found substantial variation between species (*e.g*., Pardo *et al*., 2000; Umdale *et al*., 2017; Zhao *et al*., 2016). Many different pollen types exist, and are often associated with specific plant groups, such as bisaccate pollen in conifers and monosulcate pollen of cycads (Na *et al*., 2015). These characteristics allow for long-term stability of pollen characters, and thus allowing for comparing present day pollen to fossilized pollen.

Legume pollen has been widely studied, and substantial interspecific pollen morphological variation has been found in many studies (González-Vázquez *et al*., 2017; Gunes and Aytug, 2010; Pardo *et al*., 2000; Umdale *et al*., 2017; Zhao *et al*., 2016). As Fabaceae is such an extremely species rich family, it is not too surprising that much pollen morphological variation has been recorded. For example, *Duparquetia orchidacea* Baill. is the only species in the newly described subfamily Duparquetioideae (LPWG, 2017), and has a unique pollen morphology (Banks *et al*., 2006). The asymmetrical pollen grains of this species are unique among the legumes in only having one ecto-aperture and two endo-apertures (Banks *et al*., 2006). It is further unique in the entire order Fabales, and possible within all eudicots, in having the ecto-apertures positioned latitudinally (Banks *et al*., 2006). Most other legume subfamilies have three apertures, although pollen shape and surface texture differ greatly between genera and species (Banks *et al*., 2006; Pardo *et al*., 2000; Umdale *et al*., 2017; Zhao *et al*., 2016). Few studies have focused on pollen in the tribe Genisteae, with the most examples coming from Mediterranean species (*Cytisus* Desf. – Pardo *et al*., 2000; *Genista* L. – Rizzi Longo *et al*., 2006; Rizzi Longo and Feoli Chiapella, 2009, 2007; *Retama* Raf. – Ferrauto *et al*., 2015; *Ulex* L. – Cubas and Pardo, 1992). It has been suggested that the Genisteae pollen morphology is relatively homogeneous (Ferguson and Skvarla, 1981), but substantial morphological differences in terms of grain size, ornamentation and wall structure have been related to the effects of polyploidy and flower size (Pardo *et al*., 2000). Unfortunately, there has been a lack in pollen studies focused on South African legumes, including the Genisteae. Most of the studies that included South African species were really focused on the palynology of large global genera, with some representation in South Africa. Examples include the palynological review of *Indigofera* L. by Zhao *et al*. (2016) and of *Vigna* Savi by Umdale *et al*. (2017).

Plant anatomy has proven to be of substantial systematic significance among angiosperms (Jooste *et al.*, 2016; Lauterbach *et al*., 2016; Matias *et al*., 2007; Moteetee *et al*., 2002). Leaf anatomy has proven particularly useful, and offers systematic significant traits such as mesophyll structure, shape and position of stomata (Jooste *et al*., 2016) and arrangement of vascular bundles. Lauterbach *et al*. (2016) used three leaf anatomical traits in Zygophyllaceae to show the evolution of traits beneficial to arid environments, comparing both phylogenetic and habitat perspectives. Interestingly, this study found multiple shifts in leaf traits, with leaf traits distributed unevenly across the phylogeny (Lauterbach *et al*., 2016). A similar study was undertaken in the Portulacaceae (Ocampo *et al*., 2013). In contrast, they found leaf anatomical traits, especially arrangement of vascular bundles, to very strongly track the phylogeny.

The arrangement and extent of vascular bundles in petioles have often been used to distinguish between genera and species (Matias *et al*., 2007; Olowokudejo, 1987), and even assisted in infra-generic classifications (Dehgan, 1982). Some authors have suggested petiole anatomical traits to be most useful at the generic level (Howard, 1962; Kocsis and Borhidi, 2003), although many studies have found substantial interspecific differentiation, while some families appear to have little to no anatomical variation (Kocsis and Borhidi, 2003). Petiole anatomy has also been studied fairly widely in legumes, and delivered a number of defining traits at the generic level (van Wyk and Schutte, 1989), infra-generic level (*Baphia* Lodd. – Soladoye, 1982) and specific level (*e.g. Colophospermum mopane* Benth. – Potgieter and Wessels, 1998; *Astragalus* L. – Al-Joboury, 2016; *Onobrychis* Mill. – Amirabadizadeh *et al*., 2015; *Aeschynomene* L. – Leme and Scremin-Dias, 2014). Van Wyk and Schutte (1989) studied petiole anatomy of some *Polhillia* species, but did not include all of the species now recognized in the genus.

3. CONSERVATION

The need for plant conservation has never been as important as it is in the modern day. Rapid urban development, agricultural expansion, habitat degradation, climate change, exotic species invasions and illegal plant collecting are all threatening the existence of native flora in South Africa, and other parts of the world (Raimondo *et al.*, 2009). One of the first steps in conservation is the assessment of the risk of extinction of species. South Africa is the first mega-diverse country to produce a complete Red List assessment for vascular plants (Raimondo *et al*., 2009). A massive 20 456 plant species and subspecies were assessed, of which roughly 13 265 species are endemic to SA, testifying to the botanical richness of this country (Raimondo *et al*., 2009). Furthermore, numerous new plant species have been described since the 2009 assessment, most of which are also of conservation concern (*e.g*., Curtis *et al*., 2013; Stirton and Muasya, 2016). The GCFR, with over 11 000 plant species and roughly 70 percent endemism is particularly at risk of species extinction (Manning and Goldblatt, 2012; Snijman, 2013). Manning and Goldblatt (2012) noted that roughly 38 species within the Core Cape Region (CCR) of the GCFR have gone extinct, although two species were subsequently rediscovered in 2016 (du Preez and Stirton, unpublished data; Ebrahim *et al*., 2016). More than 3000 plant species and subspecies in the CCR alone are listed as species of conservation concern (Raimondo *et al*., 2009). The most threatened regions within the GCFR are lowland areas, where most urban development and agriculture has taken place. Cape Town, for example, is seen by many as the extinction capital of South Africa due to the numerous species that are extinct or on the brink of extinction in the Cape Flats Sandstone Fynbos (Manning and Goldblatt, 2012; Raimondo *et al*., 2009). In rural areas, such as the Overberg, Swartland and Breede River Valley, large expanses of lowland vegetation (mostly Renosterveld) have been transformed into large-scale agricultural lands for grains, vineyards and deciduous fruit (Cowling *et al*., 1986; Curtis *et al*., 2013; Kemper *et al*., 1999, 2000; Raimondo *et al*., 2009). This has led to most of these unique lowland habitats and their historic biodiversity becoming degraded fragments harboring populations of highly threatened plant species. Often only small vegetation fragments remain as isolated islands in a landscape of monoculture crops (Cowling *et al*., 1986; Curtis *et al*., 2013; Kemper *et al*., 1999). Many plant species have managed to survive on these, small isolated fragments that are often the last refuges for species on the brink of extinction (Curtis *et al*., 2013; Raimondo *et al*., 2009). The effect of fragmentation over the long-term is not good, as gene flow between populations is restricted and mutualisms between pollinators and flowers are broken

(Ibáñez *et al*., 2014; Kemper *et al*., 1999). This is specifically the case for plants that rely on specialist pollinators such as long-tongue flies that have been lost from vegetation fragments resulting in some species no longer being able to reproduce sexually (Bond, 1994; Pauw, 2007). It is therefore vital for these fragments to be conserved, and restoration efforts should focus on creating corridors between vegetation fragments (Damschen *et al*., 2006; Kemper *et al*., 1999).

Polhillia is recognized as the third most threatened plant genus in SA, ranking only below *Marasmodes* and *Encephalartos* Lehm. (Magee *et al*., 2017; Raimondo *et al*., 2009). *Marasmodes,* the most threatened plant genus in SA, is coincidentally also a Renosterveld endemic with about 13 highly threatened and range-restricted species (Magee *et al*., 2017). All eight *Polhillia* species are range-restricted, and most are only present on small Renosterveld vegetation fragments (Curtis *et al*., 2013; Manning and Goldblatt, 2012; Raimondo *et al*., 2009; Snijman, 2013). As such, all species are currently listed as being of conservation concern, with statuses ranging between Critically Endangered and Vulnerable (Raimondo *et al*., 2009). The rediscovery of *Polhillia ignota* in 2016 has resulted in this species being downgraded from Extinct to Critically Endangered (Ebrahim *et al*., 2016). The plants may have been more widespread pre-agricultural development, or at least occurred as subpopulations in an unfragmented landscape. *Polhillia ignota*, for example, is now only known from a single small fragment of Swartland Shale Renosterveld that is about 4 km^2 in size, but this species has a historical distribution ranging from Vredenburg to Porterville (Ebrahim *et al*., 2016). Roughly 98 percent of its historical distribution range has been transformed for primarily wheat cultivation (Ebrahim *et al*., 2016). *Polhillia pallens* appears to be the most common and widespread species, although subpopulations are also isolated due to fragmentation, and if this species were to be split, the Red List status would likely change. It is possible that new populations of the various species may be discovered during the extensive fieldwork phase of this study, and in this way new information on metapopulation sizes and distribution will be gathered leading to updating the Red List status of all *Polhillia* species.

4. SOIL NITROGEN FIXATION

The ability to fix atmospheric nitrogen (N) into the soil for plants to use is reserved to only a few plant families in symbiosis with various types of bacteria (Benson and Silvester, 1993; Gualtieri and Bisseling, 2000). Fixation takes place in root nodules that form when bacteria infect and mitotically activate the formation of a nodule primordia (Gualtieri and Bisseling, 2000), except in Gunneraceae where the infection by *Nostoc* cyanobacteria take place in existing glands on the stems (Bergman *et al*., 1992). It is thought that the evolution of nodules happened due to a rapid increase in global temperatures and significant decreases in carbon dioxide levels, potentially limiting N in the soil, thus favoring the evolution of plants with nodulation (Sprent, 2007). Legumes are arguably the best-known N fixing family in the plant kingdom. The gram-negative bacteria that nodulate legumes, and *Parasponia* Micq. in the family Cannabaceae (Appleby *et al*., 1983), are collectively called rhizobia (Gualtieri and Bisseling, 2000). Apart from the legumes, at least eight other angiosperm families have the ability to form symbiotic relationships with N-fixing bacteria, namely Betulaceae, Casuarinaceae, Coriariaceae, Datiscaceae, Elaeagnaceae, Myricaceae, Rhamnaceae and Rosaceae (Baker, 1982; Baker and O'Keefe, 1984; Chaudhary and Mirza, 1987; Longeri and Abarzua, 1989). These families, however, all rely on gram-positive bacteria such as *Frankia* for N fixation (Benson and Silvester, 1993).

Phylogenetic data suggest that legumes and nodulation evolved roughly 60 million and 58 million years ago, respectively (Sprent, 2007). Most legumes in the subfamily Papilionoideae and many in the Mimosoideae are able to nodulate, while nodulation is less common in the Caesalpinioideae (Sprent, 2007; Sprent *et al*., 2013; Walker *et al*., 2015). Legumes in many systems act as pioneer species, stabilizing and enriching the soil with N after disturbance to allow large, longer-lived species to establish (Cocks and Stock, 2001; Power *et al*., 2010). This is especially prevalent in nutrient-poor soils such as in the fynbos, whereas in their study, Lemaire *et al.* (2015) found all studied species to nodulate within the fynbos habitat. The acidic and oligotrophic nature of soils in the CFR have meant that legumes must nodulate in order to provide usable soil nitrogen for themselves and subsequently such nitrogen is available to other plants (Power *et al.*, 2010).

Rhizobia are classified into two groups; α-proteobacteria and β-proteobacteria (Gupta, 2000; Masson-Boivin *et al*., 2009; Sawada *et al*., 2003; Walker *et al*., 2015). Knowledge of rhizobial diversity has increased considerably over the past few decades. The α-

proteobacteria first only included the genus *Rhizobium* Frank (1889), but it has now been split into multiple genera (Masson-Boivin *et al*., 2009; Willems, 2006). The recent discovery of widespread and diverse *β*-proteobacteria such as *Burkholderia* Yabuuchi *et al.* (1993) has further added to knowledge of legume-bacteria symbiosis in biological N fixation (Gyaneshwar *et al*., 2011; Lemaire *et al*., 2015; Masson-Boivin *et al*., 2009; Walker *et al*., 2015; Willems, 2006). Rhizobial diversity has been extensively studied in a variety of Cape legumes (Dludlu *et al*., 2018a, 2018b; Garau *et al*., 2009; Howieson *et al*., 2013; Kanu and Dakora, 2012; Kock, 2004; Lemaire *et al*., 2015; Steenkamp *et al*., 2015). It is clear that a large diversity of rhizobia are associated with Cape legumes, with distinct patterns of rhizobial preference between different legume groups (Lemaire *et al*., 2015). There is, however, evidence of promiscuity, with some legume tribes such as the Psoraleeae, Crotalarieae and Indigofereae (Lemaire *et al*., 2015) nodulating with both *α*- and *β*-proteobacteria (Kanu and Dakora, 2012). Although not a very diverse tribe in the CFR, Genisteae was under-represented in the Lemaire *et al*. (2015) study, with only three *Argyrolobium* species included. From these limited data, it appears that *α*-proteobacteria are favoured by Genisteae (Beukes *et al*., 2016; Bourebaba *et al*., 2016; Lemaire *et al*., 2015; Vinuesa *et al*., 2005). Specifically, the genus *Bradyrhizobium* Jordan (1982) has been recorded in European Genisteae (Bourebaba *et al*., 2016; Vinuesa *et al*., 2005), while *Mesorhizobium* as well as *Bradyrhizobium* have been found associated with *Argyrolobium* in the CFR and the SA Great Escarpment, respectively (Beukes *et al*., 2016; Lemaire *et al*., 2015). Considering the limited number of Genisteae species investigated, it is important to identify the rhizobia that nodulate the various *Polhillia* species, not only to partially fill the knowledge gap that exists in SA Genisteae species, but also for *ex situ* conservation efforts such as cultivation for reintroduction or propagation in botanical gardens. This study will include the first large scale propagation effort of all *Polhillia* species using field-collected seed and native soils to give the best chance of successful propagation and long-term survival of plants. This will also allow for the harvest of rhizobia as mature plants in the field rarely have rhizobia and to prevent the loss of wild individuals.

5. SOIL NUTRIENTS

Soil type and nutrient characters vary in the landscape and often lead to the localization of species (Cowling, 1990; Cowling and Holmes, 1992). The type of soil, soil pH and the concentrations of macro-nutrients, especially nitrogen (N) and phosphorus (P), greatly

influence the distribution of plant species and vegetation community boundaries (Richards *et al*., 1997; Thwaites and Cowling, 1988; Witkowski and Mitchell, 1987). Other important and variable cations often analyzed in soils include calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) (Richards *et al*., 1997). The Renosterveld vegetation grows on fairly fertile duplex soils derived from shale deposits (Cowling and Holmes, 1992; Kemper *et al*., 1999; Thwaites and Cowling, 1988). The soils are relatively low in P with a mean of 9.7 mg/kg measured by Kemper *et al*. (1999), although this would vary across the landscape. These concentrations of P are, however, substantially higher than in other soils in the CFR that are typically home to fynbos vegetation (Witkowski and Mitchell, 1987). The shale-derived soils on which Renosterveld grows also have a higher soil pH (5.9–6.9; Solomon, 2015) than the very acidic, low pH sandstone soils that are home to fynbos (3.9–4.3) (Mitchell *et al.*, 1984). Although this pH range is ideal for plant growth (Amacher *et al*., 2007), it cannot be viewed as the general reading for all Renosterveld vegetation. This measurement was taken near Nieuwoudtville, which is far removed from all known *Polhillia* sites. Dludlu *et al*. (2018c) studied legume assemblage distribution in different soils on the Cape Peninsula, and showed patterns of legume assemblage soil specificity. Within an edaphically heterogeneous landscape based on multiple soil parameters, legume species assemblages were often restricted to very specific soil conditions (Dludlu *et al*., 2018c). This forms a solid baseline study upon which other soil parameter specificity studies of related legumes elsewhere in the GCFR can built. The distribution patterns of *Polhillia* species, especially in the Overberg, and scattered into similar habitats within the CFR, are puzzling. The historical distribution of the various species, before the advent of agriculture, will never be known, but the distributions of at least three species appear to overlap. There is, however, no evidence of any two or more species growing together. The Overberg species grow in Rûens Shale Renosterveld, *Polhillia ignota* grows in Swartland Shale Renosterveld, *Polhillia obsoleta* grows in Breede Alluvium Renosterveld and *Polhillia involucrata* grows in Roggeveld Shale Renosterveld (Mucina and Rutherford, 2006). Very little data are available on soil nutrients and characters in Renosterveld vegetation in general, with the few past studies having been confined to highly localized areas (Kemper *et al*., 1999; Solomon, 2015).

6. AIMS AND OBJECTIVES OF STUDY

This thesis is a culmination of a broad scale investigation into various aspects of the highly threatened Cape legume genus, *Polhillia*. The thesis is split into three data chapters, each with a single respective aim and specific objectives listed below:

Aim 1: Test the monophyly of the genus *Polhillia*

Objective 1: Construct robust molecular phylogenies using different markers.

Aim 2: Conduct a systematic revision of the genus *Polhillia*

Objective 1: Use morphological, palynological, anatomical and molecular phylogenetic data to revise species boundaries.

Objective 2: Describe potential new taxon proposed by Stirton (*pers. comm.*) and any other species potentially found during the study.

Objective 3: Conduct extensive fieldwork to collect samples and gather population data of all *Polhillia* species across as many populations as possible.

Objective 4: Revise the conservation status of all species using categories and criteria of the IUCN based on the planned extensive fieldwork.

Aim 3: Investigate ecophysiological factors that may influence the distribution of *Polhillia* species

Objective 1: Investigate and compare habitat and soil parameter profiles of all *Polhillia* species in order to evaluate their influence on species specific distributions.

Objective 2: Investigate rhizobial symbiont diversity of all *Polhillia* species.

Objective 3: Determine whether the distribution of *Polhillia* species is influenced by rhizobial symbiont preferences.

7. BREAKDOWN OF CHAPTERS

Chapter 2

In this chapter I tested the monophyly of the genus *Polhillia* and consider the generic boundaries based on all included species (recognized in Chapter 3). The new monotypic subgenus *Roseopolhillia* was described.

This chapter has been prepared for publication in the South African Journal of Botany (SAJB).

Chapter 3

This chapter presents a multidisciplinary monograph in which I revise the genus *Polhillia*. We use numerous morphological characters to provide detailed morphological species descriptions. All species are accompanied by a composite photographic plate illustrating key characters for species level identification, etymology, a description of habitat, ecology and distribution (along with a map), and the conservation assessment according to the IUCN categories and criteria.

This chapter has been prepared for publication in the South African Journal of Botany (SAJB).

Chapter 4

The role of habitat preferences, soil type differences and diversity of rhizobial symbionts in shaping the distribution of extant *Polhillia* species are compared in Chapter 4. Detailed habitat parameters such as slope aspect, gradient, underlying geology and landscape topology as well as soil profiles from multiple sites per species were investigated to determine soil specific preferences of the different species. This was then used as explanation of why *Polhillia* species never co-occur in sympatry. Rhizobial symbionts were further isolated from seedlings grown under greenhouse conditions in their native soils. In addition to providing valuable information about the symbiont diversity associated with members of the Genisteae, results also suggest that rhizobial preferences seems to have been relatively unimportant as driver of radiation in this genus.

This chapter has been prepared for publication in Journal of Plant Ecology.

Chapter 5

This chapter draws together the final conclusions of the entire thesis, in answer to the aims set above.

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Declaration by the candidate (for chapter prepared for South African Journal of Botany)

With regards to Chapter 2 (**THE GENERIC CONCEPT OF *POLHILLIA* C.H.STIRT. (GENISTEAE: FABACEAE): CONFIRMATION OF THE MONOPHYLY OF THE GENUS AND DESCRIPTION OF A NEW MONOTYPIC SUBGENUS *ROSEOPOLHILLIA***), the nature and scope of my contribution were as follows:

Nature of contribution	**Extent of contribution (%)**
Data collection, analysis, interpretation and manuscript preparation	80

The following co-authors have contributed to Chapter 2:

Name	**e-mail address**	**Nature of contribution**	**Extent of contribution (%)**
Léanne L. Dreyer	ld@sun.ac.za	Provided guidance and edited the manuscript	10
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Signature of candidate: .....…Brian du Preez…….

Declaration by co-authors:

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 2.
2. No other authors contributed to Chapter 2 than those specified above.
3. There are no conflicts of interest relevant to Chapter 2 of this dissertation.

Signature	**Institutional affiliation**	**Date**
Léanne L. Dreyer	Stellenbosch University	December 2018
Muthama Muasya	UCT	December 2018

CHAPTER 2

THE GENERIC CONCEPT OF *POLHILLIA* C.H.STIRT. (GENISTEAE: FABACEAE): CONFIRMATION OF THE MONOPHYLY OF THE GENUS AND DESCRIPTION OF A NEW MONOTYPIC SUBGENUS *ROSEOPOLHILLIA*

ABSTRACT

Polhillia is a small, fairly recently described legume genus endemic to the Greater Cape Floristic Region (GCFR) of South Africa. The genus was initially described to accommodate species erroneously placed in other Cape legume genera. Further new *Polhillia* species were later discovered and described. The relationships among species and monophyly of the genus has, however, not been tested. We aimed to test this using both morphological and molecular evidence. We confirmed *Polhillia* to indeed be a natural entity that resolved to be monophyletic in the molecular phylogenetic reconstruction, with one morphologically distinct species, *Polhillia involucrata*, resolving as sister to the rest of the genus. Internal phylogenetic resolution was weak and clades either displayed low probability values or several polytomies were present. Based on total evidence data, we thus circumscribe *Polhillia involucrata* into the new monotypic subgenus *Roseopolhillia*.

1. INTRODUCTION

The genus *Polhillia* C.H.Stirt. was first described in 1986 based on a set of unique traits, including the fusion of stipules that sheathe the stem (Stirton, 1986a). Initially only four species (*P. waltersii* (C.H.Stirt.) C.H.Stirt., *P. connata* (Harv.) C.H.Stirt., *P. canescens* C.H.Stirt. and *P. pallens* C.H.Stirt.) were placed in the genus (Stirton, 1986a). A fifth species informally referred to as *Polhillia sp. A* in Stirton (1986a), was later described as *Polhillia ignota* Boatwr. (Boatwright, 2010). Van Wyk and Schutte (1989) moved a further two species (*Polhillia brevicalyx* (C.H.Stirt.) B.-E.van Wyk & A.L.Schutte and *Polhillia involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte) to *Polhillia* from other genera. *Polhillia waltersii* was later synonymized under *Polhillia obsoleta* (Harv.) B.-E.van Wyk (van Wyk, 1992). *Polhillia brevicalyx* was originally described under the genus *Argyrolobium* Eckl. & Zeyh., but was found to be morphologically better placed in *Polhillia* (Stirton, 1984; van Wyk and Schutte, 1989). *Polhillia involucrata*, a very unique species, has been moved between several genera since its original description as *Psoralea involucrata* Thunb. Stirton (1986b) reluctantly included it in *Melolobium* Eckl. & Zeyh., but based on anatomical, alkaloid (van Wyk *et al*., 1988) and morphological data, van Wyk and Schutte (1989) proposed its inclusion in *Polhillia*. Unfortunately, their analyses considered very distantly related species from the tribe Crotalarieae as outgroups and neglected to mention several key differences between *Polhillia involucrata* and the rest of the genus. There has also been confusion about flower colour of *Polhillia involucrata*, with some authors suggesting that the flowers open yellow and fade to purple with age (Snijman, 2013; van Wyk and Schutte, 1989).

The taxonomy of southern African Fabaceae has changed considerably over the past few decades. When *Polhillia* was revised by van Wyk and Schutte (1989), *Polhillia*, *Melolobium* and *Dichilus* DC. were still placed in the tribe Crotalarieae, while only *Argyrolobium* was recognized as belonging to the tribe Genisteae (Polhill, 1976). A better understanding of the morphological characters defining the tribe Genisteae, along with the dawn of molecular phylogenetics, has resulted in improved circumscription of the tribe (Cardoso *et al*., 2013; Crisp *et al*., 2000; Lewis *et al*., 2005; LPWG, 2017). Although the taxonomy of most included members of the SA Genisteae has been revised in recent years (Edwards, 1994; Moteetee and van Wyk, 2006; Schutte and van Wyk, 1988), the taxonomy of *Polhillia* has remained neglected and most of the species remained poorly known.

Following the systematic revision of the genus (du Preez *et al*., 2019 – Chapter 3), the following 11 species are now recognized in the genus *Polhillia*:

- *Polhillia brevicalyx* (C.H.Stirt.) B.-E.van Wyk & A.L.Schutte
- *Polhillia connata* (Harv.) C.H.Stirt.
- *Polhillia curtisiae* C.H.Stirt. & Muasya
- *Polhillia fortunata* B.du Preez *sp. nov.*
- *Polhillia groenewaldii* B.du Preez *sp. nov.*
- *Polhillia ignota* Boatwr.
- *Polhillia involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte
- *Polhillia obsoleta* (Harv.) B.-E.van Wyk
- *Polhillia pallens* C.H.Stirt.
- *Polhillia stirtoniana* B.du Preez *sp. nov.*
- *Polhillia xairuensis* B.du Preez *sp. nov.*

There are several morphological characters that support *Polhillia* as a natural entity, but the inclusion of the morphologically obscure *Polhillia involucrata* (Figure 1), without molecular phylogenetic data, brings the monophyly into question. No wide scale molecular phylogenetic analyses have been done on the genus, with a few studies using *Polhillia* samples as outgroups for broader molecular phylogenies (Boatwright *et al*., 2008; Swanepoel *et al*., 2015). This study aims to test the monophyly of *Polhillia* based on molecular phylogenetic data, while also investigating morphological synapomorphies between all species.

Figure 1: Front view of flowers of all *Polhillia* species recognised by du Preez *et al*. (2019–Chapter 3): 1 – *P. brevicalyx*; 2 – *P. connata*; 3 – *P. groenewaldii*; 4 – *P. curtisiae*; 5 – *P. fortunata*; 6 – *P. ignota*; 7 – *P. obsoleta*; 8 – *P. pallens*; 9 – *P. stirtoniana*; 10 – *P. xairuensis*; 11 & 12 – *P. involucrata* (pink and white forms respectively). Photographs by B. du Preez.

2. MATERIALS AND METHODS

2.1. Morphology

Morphological data was obtained from fresh material collected during extensive fieldwork and was supplemented by studying specimens from the following herbaria: BOL, NBG, PRE, K, and JRAU (abbreviations according to Thiers (2017). Morphological character descriptions and measurements made by du Preez *et al*. (2019 – Chapter 3) are used.

2.2. Molecular phylogenetic reconstruction

Total DNA was extracted from silica dried leaf material using the CTAB method (Doyle and Doyle, 1987). Although I collected most of the material in the field, additional material was provided by Prof. C.H. Stirton and Dr. J.S Boatwright. Specimens used for the molecular phylogenetic reconstruction of *Polhillia* are summarized in Table 1. Two northern hemisphere Genisteae sequences (Table 2) were also downloaded from GenBank and included as outgroup taxa. Polymerase Chain Reactions (PCR) were run using nuclear Internal Transcribed Spacer (ITS) and plastid (trnS-trnG, psbA-trnH) markers. For ITS, the universal AB101 and AB102 primer pair was used (Sun *et al*., 1994) and the reaction was run using the following protocol: initial denaturation at 94°C for 5 min; 35 cycles of 94˚C for 1 min, 61˚C for 1 min and 72˚C for 90 s, respectively; final extension for 6 minutes at 72˚C. For the trnS-trnG region, we used the trnS(GCU) and trnG(UUG) primer pair of Hamilton (1999) using the following protocol: initial denaturation for 5 min at 96°C; 35 cycles of 96°C for 45 s, 52°C for 1 min, 72°C for 1 min; final extension at 72°C for 7 min. Primers for the psbA-trnH region are specified in Hamilton (1999) and PCR protocol followed was 95 for 3 min; 35 cycles of 95 for 30 s, 55 for 1 min, 72 for 90 s; 72 for 4 min (Sang *et al*., 1997). Direct sequencing was done at the Stellenbosch Central Analytical Facility (CAF). Sequences were edited using Chromas2 v2.3 (Technelysium Pty Ltd, 2004), and were aligned using BioEdit v7.2.5 (Ibis Biosciences, 2013). Phylogenetic analyses were done through Bayesian Inference (BI) using MrBayes v3.2 (Ronquist and Huelsenbeck, 2003) under nst=mixed model of nucleotide substitution and an invgamma parameter to account for among site rate variation, for 10^6 generations and trees were sampled every 2000 generations under otherwise standard settings. All runs reached stationarity on the posterior as judged by MrBayes internal diagnostics. Phylogenetic consensus trees were viewed and analyzed using FigTree v1.4.3 (Rambaut, 2009).

Table 1: *Polhillia* and outgroup samples used for molecular phylogenetic analysis through DNA extraction and sequencing.

Species			**Regions sequenced**		
	Collector and number	Herbarium	ITS	trnS-G & psbA-trnH	Combined Nuclear & Plastid
Argyrolobium_amplexicaulis_6758	Muasya 6758	BOL	X		
Argyrolobium_argenteum_486	BdP486	BOL	X		
Argyrolobium_harmsianum_386	BdP386	BOL	X	X	X
Argyrolobium_molle_359	BdP359	BOL	X	X	X
Argyrolobium_pachyphyllum_304	BdP304	BOL	X	X	X
Argyrolobium_polyphyllum_360	BdP360	BOL	X		
Argyrolobium_tuberosum_6611	Muasya 6611	BOL	X		
Aspalathus_nov_485		BOL	X		
Dichilus_gracilis_2501	C.M. van Wyk 2501	NBG	X		
Lupinus_luteus_13907	Stirton 13907	BOL	X	X	X
Melolobium_adenodes_318	BdP318	BOL	X	X	X
Melolobium_candicans_408	BdP408	BOL	X	X	X
Polhillia_brevicalyx_280	BdP280	BOL	X	X	X
Polhillia_brevicalyx_396	BdP396	BOL	X	X	X
Polhillia_connata_287	BdP287	BOL	X	X	X
Polhillia_connata_383	BdP383	BOL	X		
Polhillia_connata_390	BdP390	BOL	X		
Polhillia_connata_519	BdP519	BOL	X	X	X
Polhillia_curtisiae_282	BdP282	BOL	X		
Polhillia_curtisiae_286	BdP286	BOL	X	X	X
Polhillia_curtisiae_334	BdP334	BOL	X		
Polhillia_curtisiae_510	BdP510	BOL	X	X	X
Polhillia_fortunata_501	BdP501	BOL	X	X	X
Polhillia_groenewaldii_522a	BdP522	BOL	X	X	X
Polhillia_groenewaldii_522b	BdP522	BOL	X	X	X

Polhillia_ignota_192	BdP192	BOL	X	X	X
Polhillia_ignota_323	BdP323	BOL	X	X	X
Polhillia_involucrata_279	BdP279	BOL	X	X	X
Polhillia_involucrata_397	BdP397	BOL	X	X	X
Polhillia_involucrata_402	BdP402	BOL	X		
Polhillia_involucrata_409	BdP409	BOL	X	X	X
Polhillia_obsoleta_277	BdP277	BOL	X	X	X
Polhillia_obsoleta_523	BdP523	BOL	X	X	X
Polhillia_pallens_291	BdP291	BOL	X	X	X
Polhillia_pallens_310	BdP310	BOL	X	X	X
Polhillia_pallens_319	BdP319	BOL	X		
Polhillia_pallens_512	BdP512	BOL	X	X	X
Polhillia_pallens_514	BdP514	BOL	X		
Polhillia_stirtoniana_281	BdP281	BOL	X	X	X
Polhillia_stirtoniana_309	BdP309	BOL	X	X	X
Polhillia_stirtoniana_516	BdP516	BOL	X	X	X
Polhillia_stirtoniana_517	BdP517	BOL	X		
Polhillia_xairuensis_315	BdP315	BOL	X	X	X
Polhillia_xairuensis_337	BdP337	BOL	X	X	X
Polhillia_xairuensis_392	BdP392	BOL	X		
Spartium_junceum_14120	Stirton 14120	BOL	X	X	X

Table 2: Samples downloaded from GenBank and used for molecular phylogenetic reconstruction.

Region	**Species**	**GenBank No.**	**Reference**
ITS	*Anarthrophyllum_rigidum*	FJ839488.1	Cubas *et al*. 2010
	Adenocarpus_nainii	FJ839481.1	Cubas *et al*. 2010

3. RESULTS

3.1. Morphology

Several morphological traits were identified that define members of *Polhillia* and distinguish them from all other South African (SA) genera in the tribe Genisteae. Unique generic traits include the absence of true peduncles and flowers that are single, in terminal clusters or pseudo-pedunculate. Other SA genera typically have flowers borne on true peduncles, except for a few *Argyrolobium* species. *Polhillia* taxa have two stipules that are fused, at least around the base, and thus sheath the stem. Other genera typically have two unfused stipules that do not sheath the stem. Most *Polhillia* taxa have subsessile leaves, while other genera typically have distinctly petiolate leaves. *Polhillia* species also lack bracteoles, which are common in all other SA Genisteae genera. Imbricate keel petals are also a unique character shared among *Polhillia* species and is absent from all other SA Genisteae genera. Despite these strong morphological traits that support the delimitation of *Polhillia s.l.*, morphological data also highlighted the uniqueness of *Polhillia involucrata* within this genus. It has several morphological features that differ from these typical generic *Polhillia* traits. (Table 3).

Table 3: Prominent morphological differences between *P. involucrata* and other *Polhillia* species.

Character	***P. involucrata***	**Other *Polhillia* species**
Flower colour	Pink or white	Yellow
Pod	Dehiscent	Indehiscent
Leaf and stipule indumentum	Leaves mostly glabrous or sparsely pilose. Stipules abaxially glabrous.	Leaves and stipules densely pubescent.

3.2. Molecular phylogenetic reconstruction

Nuclear (ITS) and combined plastid (trnS-G and psbA-trnH) consensus trees created through Bayesian inference are presented in Figures 2 and 3, while a combined nuclear and plastid tree is presented in Figure 4. All three phylogenetic reconstructions confirm *Polhillia* as a natural monophyletic genus with 1.0 probability. *Polhillia involucrata* also resolves as sister to the rest of the genus with 1.0 probability in all three phylogenetic reconstructions (Figures

2–4).. This represents strong support when considering the poor internal phylogenetic resolution within the rest of the genus *Polhillia*. Individual nuclear (ITS) and plastid (trnS-G & psbA-trnH) trees are largely unresolved, or when clades are defined, they are poorly supported. The combined nuclear and plastid tree provides relatively good internal phylogenetic resolution, except in an unresolved clade comprised of *P. curtisiae*, *P. pallens* and *P. xairuensis*.

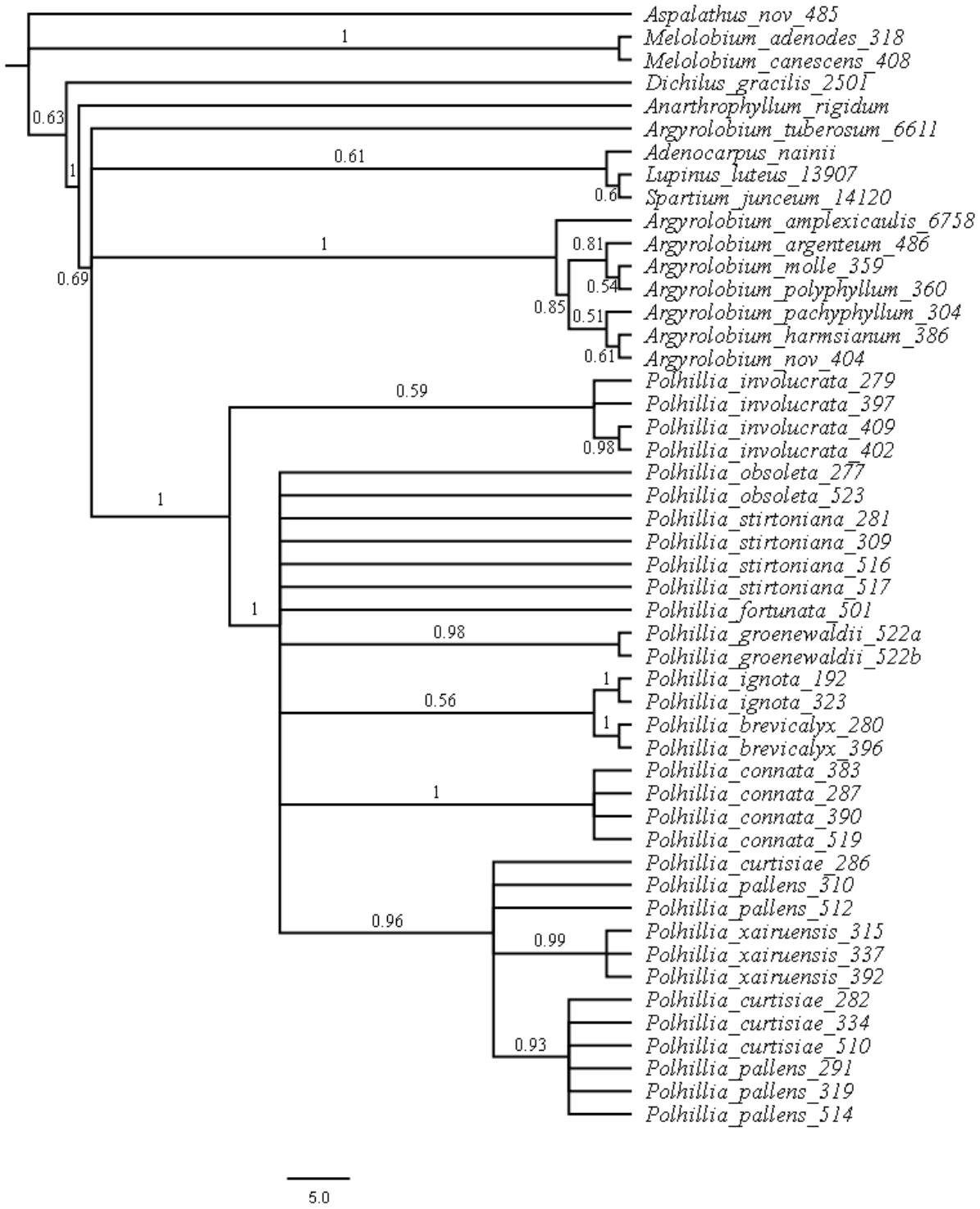

Figure 2: Nuclear ITS phylogeny of *Polhillia* and other Genisteae relatives inferred through Bayesian Inference in Mr Bayes. Values above branches indicate posterior probabilities. Trees were run for 10 million generations and sampled every 2000 generations**.**

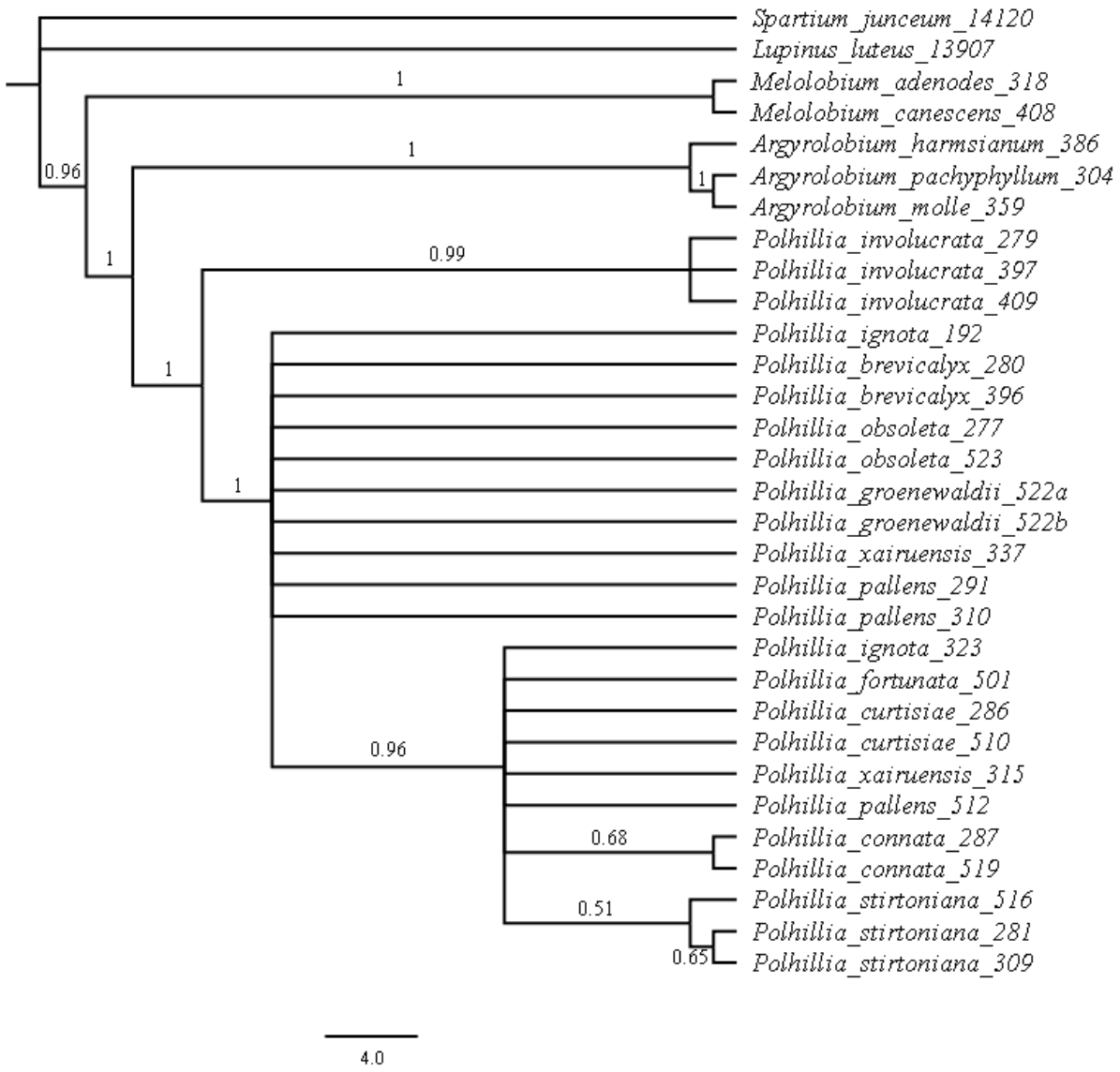

Figure 3: Combined plastid trnS-trnG and psbA-trnH phylogeny of *Polhillia* and other Genisteae relatives inferred through Bayesian Inference in Mr Bayes. Values above branches indicate posterior probabilities. Trees were run for 10 million generations and sampled every 2000 generations.

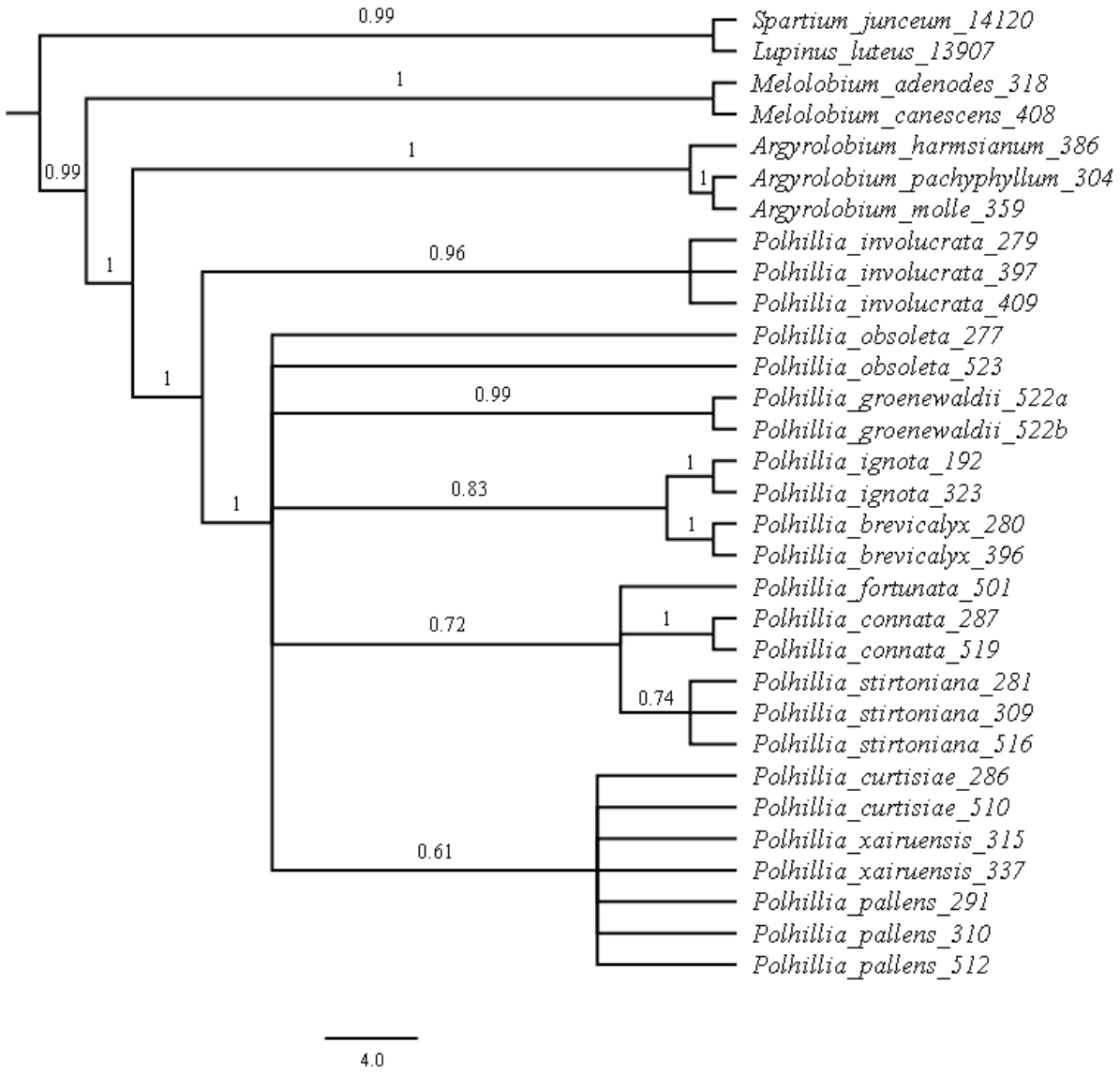

Figure 4: Combined nuclear (ITS) and plastid (trnS-G; psbA-trnH) phylogeny of *Polhillia* and Genisteae outgroups inferred through Bayesian Inference in Mr Bayes. Values above the branches indicate branch posterior probabilities. Trees were run for 10 million generations and sampled every 2000 generations.

4. DISCUSSION

Polhillia is a fairly recently described genus, instated initially to include species removed from the genera *Argyrolobium* and *Lebeckia* as well as to describe two new species (Stirton, 1986a). Two additional species were included into the genus by van Wyk and Schutte (1989), while two further new species had been described when this study commenced (Boatwright, 2010; Curtis *et al*., 2013). It was, therefore, important to investigate whether represents a monophyletic genus. The description of *Polhillia* by Stirton (1986a) was based also on biochemical data, which showed that *Polhillia* has fairly distinctive biochemical properties compared to other Genisteae genera. The biochemical profile of *Polhillia involucrata* was found to resemble members of *Melolobium*, which led Stirton (1986b) to include it in *Melolobium*, rather than in *Polhillia*. Results from other morphological studies (Moteetee *et al*., 2002; van Wyk and Schutte, 1989) on SA Genisteae genera provided further support for *Polhillia* as a distinct genus. Van Wyk and Schutte (1989) used a combination of anatomical and morphological characters to broaden the concept of *Polhillia*, further supported by alkaloid data (van Wyk *et al*., 1988). They found petiole anatomy, along with flower and leaf morphology to support their broader concept of *Polhillia*, a view we share. Their study was, however, based on a very small sample size for most included species, which has been shown to be problematic. Although they noted the dehiscent pods and pink flowers of *Polhillia involucrata*, van Wyk and Schutte (1989) did not view these variant traits as sufficient justification to exclude this species from *Polhillia*. They, however, did not consider subgeneric reclassification.

We found *Polhillia* species to share a unique suite of morphological traits, which include fused stipules that sheathe the stem, imbricate keel petals, absence of bracteoles, and absence of true peduncles, with several species having pseudo-peduncles (du Preez *et al*., 2019 – Chapter 3). We deem these characters diagnostic enough to confirm the genus as a natural entity. Most *Polhillia* species are morphologically very similar, which at times complicated species circumscription (du Preez *et al*., 2019 – Chapter 3). *Polhillia involucrata* fits the broad concept of *Polhillia* proposed by van Wyk and Schutte (1989), as a true peduncle is absent and stipules are connate and sheathe the stem. It differs, however, in having pink or rarely white flowers and dehiscent pods. As flower colour is typically not variable within genera of GCFR legumes (Campbell and van Wyk, 2001; Dahlgren, 1988; Kies, 1951; Manning and Goldblatt, 2012; Schutte-Vlok and van Wyk, 2011), it is a significant trait within the context of SA Genisteae (Edwards, 1994, 2005; Moteetee and van Wyk, 2006;

Schutte and van Wyk, 1988; Stirton, 1986a). *Polhillia involucrata* is the only known pink-flowered species among all SA Genisteae species as all other species are either yellow to orange or very rarely white-flowered (Edwards, 2005, 1994; Moteetee and van Wyk, 2006; Schutte and van Wyk, 1988; Stirton, 1986a). Legume genera in the GCFR are in general relatively conserved in colour (Manning and Goldblatt, 2012), although there are exceptions. *Aspalathus* L. for example is typically yellow flowered, but has several unrelated shifts in colour to different shades of purple, pink and white (Dahlgren, 1988).

Polhillia involucrata is the only species in the genus with dehiscent fruit. This trait is typical for most SA Genisteae, rendering the indehiscent fruit of all other *Polhillia* species unusual in this tribe (Edwards, 2005, 1994; Moteetee and van Wyk, 2006; Schutte and van Wyk, 1988; Stirton, 1986a). Dehiscence is believed to be the ancestral state among angiosperms, and indehiscence is known to have had multiple independent origins (Beaulieu and Donoghue, 2013). In the mega diverse Campanulidae angiosperm clade, consisting of over 35 000 species globally, the majority of species have indehiscent fruits with dehiscence becoming a rare ancestral state (Beaulieu and Donoghue, 2013). The wide scale analysis of Beaulieu and Donoghue (2013) also indicated that the shift to indehiscence is one-directional as there was no evidence of a reverse shift back to dehiscent fruit. The two Magnoliaceae subfamilies are, for example, split on the basis of fruit dehiscence (Romanov and Dilcher, 2013). Multiple independent shifts from dehiscence to indehiscence have also been shown to be phylogenetically significant in the Brassicaceae (Mühlhausen *et al*., 2013). Among GCFR legumes, dehiscence appears to be the favoured fruit type although there are some genera that have evolved indehiscent fruit (Boatwright *et al*., 2009; Dahlgren, 1975; Manning and Goldblatt, 2012). *Wiborgia* Thunb. is one of the few Cape legume genera with exclusively indehiscent fruit (Boatwright *et al*., 2009; Dahlgren, 1975; Manning and Goldblatt, 2012), while *Lebeckia Thunb*., *Calobota* (Eckl. & Zeyh.) Boatwr. & B.-E.van Wyk and *Wiborgiella* Boatwr. & B.-E.van Wyk has both dehiscent and indehiscent fruit (Boatwright *et al*., 2009; Manning and Goldblatt, 2012). Despite these morphological traits that present a reasonable argument to circumscribe *Polhillia involucrata* from the genus, it does share the diagnostic traits of current *Polhillia* species such as flowers that are clustered terminally, the absence of a peduncle, presence of two fused stipules that sheath the stem, and the absence of bracteoles.

Molecular phylogenetic resolution within the genus *Polhillia* proved to be poor for both plastid and nuclear markers used, although resolution to generic level was strong. Molecular phylogenetic resolution was improved when nuclear and plastid trees were combined. Despite

the poor internal resolution within *Polhillia*, molecular phylogenetic data strongly supports morphological evidence that *Polhillia* represents a monophyletic genus. It also supports our hypothesis that *Polhillia involucrata* is sister to the rest of *Polhillia*, and deserves recognition at least at the subgeneric level. The finding of 1.0 probability for *Polhillia involucrata* as sister to the rest of *Polhillia* in each of the ITS, combined trnS-G and psbA-trnH tree and combined nuclear and plastid tree is strong evidence for subgeneric recognition. Considering the molecular evidence along with strong morphological differences including that *Polhillia involucrata* has pink or rarely white flowers and dehiscent fruit (*versus* yellow flowers and indehiscent fruit), there is strong evidence supporting the circumscription of *Polhillia involucrata* into a new monotypic subgenus. Future next generation sequencing would hopefully provide better resolution within the rest of the genus *Polhillia* to enable us to infer evolutionary patterns and determine species relations.

5. TAXONOMIC TREATMENT

5.1. Key to the genera of SA Genisteae

1a Glands usually present on flowers and leaves, stipules completely free and auriculate _____ *Melolobium*

1b Glands absent from flowers and leaves, stipules free or fused, but not auriculate _____ 2

2a Stipules highly reduced, standard and wing petals strongly reflexed _____ *Dichilus*

2b Stipules clearly visible, standard and wing petals not strongly reflexed _____ 3

3a Stipules free, broad-based to amplexicaul and not sheathing stem, bracteoles present _____ *Argyrolobium*

3b Stipules fused and sheathing stem, bracteoles absent _____ *Polhillia*

5.2. *Polhillia* subgen. *Polhillia* C.H.Stirt.

5.2.1. Taxonomy

Type species: *Polhillia waltersii* (C.H.Stirt.) C.H.Stirt. (=*Lebeckia waltersii* C.H.Stirt.) S. Afr J. Bot 52: 4 (1986). Type: Worcester commonage 1.5 miles north-west of town (–CB), 30 Jul 1975, *Rourke 1484* (K, holotype!; NBG!, isotype).

Woody shrubs, erect or spreading, sprouting from rootstock after fire; stipules 2, deltoid, pubescent, connate, sheathing stem, often persistent; leaves digitately trifoliolate, subsessile or petiolate in *P. brevicalyx*, pubescent, conduplicate; inflorescence terminal, flowers single or in clusters of 2 (or 4–5 in *P. brevicalyx*, *P. ignota* and *P. fortunata*), subsessile or pseudo-pedunculate up to 15 mm long; flowers yellow, 8–13 mm long; calyx with trifid ventral lip, villous-sericeous; standard petal broadly ovate, apex emarginate, pubescent abaxially; wing petals variable in shape and sculpturing, mostly glabrous or pilose along midrib; keel petals variable in shape and level of pubescence, usually restricted to the front edge; stamens 10, monodelphous with single dorsal slit, filaments fused for most of their length, anthers alternating between long and short; pistil sericeous or villous, ovules 7–12, style sub-erect to erect, stigma regular, capitate; pod indehiscent, linear-oblong, laterally compressed between seeds, plicate in *P. ignota*, *P. obsoleta*, *P. groenewaldii*, and *P. fortunata*, sericeous to glabrescent; seeds ovate-reniform, smooth, uniformly coloured, olive green to dark brown (except for *P. connata* and *P. pallens* that have occasional mottling).

5.2.2. Species included:

1. ***Polhillia brevicalyx*** (C.H.Stirt.) B.-E.van Wyk & A.L.Schutte, Kew Bulletin 44: 3 (1989); *Argyrolobium brevicalyx* C.H.Stirt. S, Afr. J. Bot. 50: 4 (1984). Type—South Africa, Western Cape, Flats along watercourse 1 km SSE of Uitvlug farm, along road between Bredasdorp and Swellendam, 15 km SW of Swellendam, 1 October 1983, *Burgers 3188* (K, holotype!; STE, isotype!).

2. ***Polhillia connata*** (Harv.) C.H.Stirt., S. Afr. J. Bot. 52: 2 (1986). Type—Cap. Bon. Spei, without precise locality, Thom 37 (K, holotype!). *Argyrolobium connatum* Harv., Fl. Cap. 2: 72 (1862). *Genista connata* (Harv.) Briq., Étude Cytises Alpes Mar. 119 (1894).
   =*Polhillia canescens* C.H.Stirt. S Afr. J. Bot. 52: 2 (1986). syn. nov. (Chapter 3). Type—South Africa, Western Cape, Adamskop, north of Bredasdorp, 23 September 1982, *Bayer 3104* (NBG, Holotype!).

3. ***Polhillia curtisiae*** C.H.Stirt. & Muasya, S Afr. J. Bot. 87 (2013). Type—South Africa, Western Cape, Bredasdorp Dist., Haarwegskloof farm, 12 October 2011, *Stirton, Muasya & Curtis 13361* (BOL, holotype!; NBG, isotype!).

4. ***Polhillia ignota*** Boatwr., S. Afr. J. Bot. 76 (2010). Type—South Africa, Western Cape, Between Vredenburg and "Hoetjesbaai" [Houdjies Bay], 5 September 1928, *Hutchinson 253* (K, holotype!).

5. ***Polhillia obsoleta*** (Harv.) B.-E.van Wyk, Bothalia 22: 1 (1992). *Argyrolobium obsoletum* Harv. Flora Capensis 2: 70 (1862). Type—Cape, without precise locality, *Thunberg s.n. sub UPS-THUNB 16504* (UPS, holotype). *Aspalathus sericea sensu* Thunb. Flora Capensis 574 (1823) non DC.
   =*Polhillia waltersii* (C.H.Stirt.) C.H.Stirt. S. Afr J. Bot 52: 4 (1986). *Lebeckia waltersii* C.H.Stirt. Bothalia 13: 3-4 (1981). Type—South Africa, Western Cape, Worcester commonage 1.5 miles north-west of town, 30 July 1975, *Rourke 1484* (K, holotype!, NBG, isotype!).

6. ***Polhillia pallens*** C.H.Stirt., S Afr. J. Bot. 52: 2 (1986). Type—South Africa, Western Cape, 3 km west of Breede River mouth on S side of river along access road to Kontiki, 22 February 1981, *C.J. Burgers 2633* (NBG, holotype!; K, isotype!).

7. ***Polhillia groenewaldii*** B.du Preez, *sp. nov.* (Chapter 3). Type—South Africa, Western Cape, Bonnievale Region, Bokdam farm, NW of Stormsvlei Pass, 8 August 2018, *du Preez 522* (BOL, holotype!; K, MO, PRU, NBG, STE, isotype!).

8. ***Polhillia stirtoniana*** B.du Preez, *sp. nov.* (Chapter 3). Type—South Africa, Western Cape, Bredasdorp Region, Plaatjieskraal farm, farm road leading west, 7 August 2017, *du Preez 309* (BOL, holotype!; K, MO, isotype!)

9. ***Polhillia xairuensis*** B.du Preez, *sp. nov.* (Chapter 3). Type—South Africa, Western Cape, Suurbraak Region, Voorstekop farm, 11 August 2017, *du Preez 315* (BOL, holotype!; K, MO, isotype!).

10. ***Polhillia fortunata*** B.du Preez, *sp. nov.* (Chapter 3). Type—South Africa, Western Cape, Vanwyksdorp District, Farm Waterval, about 1.5km east of homestead along road to Herbertsdale, 23 September 2018, *du Preez 564* (BOL, holotype!; K, MO, STE, NBG, PRU, PRE, SCHG, isotype!).

5.2.3. Etymology

The generic and subgeneric epithet *Polhillia* is in honour of renowned botanist and legume systematist Roger M. Polhill from the Royal KEW Botanical Gardens (Stirton, 1986a).

5.2.4. Diagnostic characters

Species in *Polhillia* subgen. *Polhillia* are distinguished from the species in *Polhillia* subgen. *Roseopolhillia* by having yellow flowers (*versus* flowers pink or rarely white), indehiscent pods (*versus* dehiscent pods), pubescent leaves and stipules (*versus* mostly glabrous leaves, and stipules that are pubescent only on their adaxial surface, glabrous on outer surface).

5.2.5. Distribution

Species in *Polhillia* subgen. *Polhillia* are distributed through lowland Renosterveld vegetation (1–350m) in the Overberg, Breede River Valley, Swartland and Little Karoo regions of the Western Cape Province (Figure 5). This subgenus is endemic to the Core Cape Region (CCR) of the Greater Cape Floristic Region (GCFR) *sensu* Manning & Goldblatt (2012).

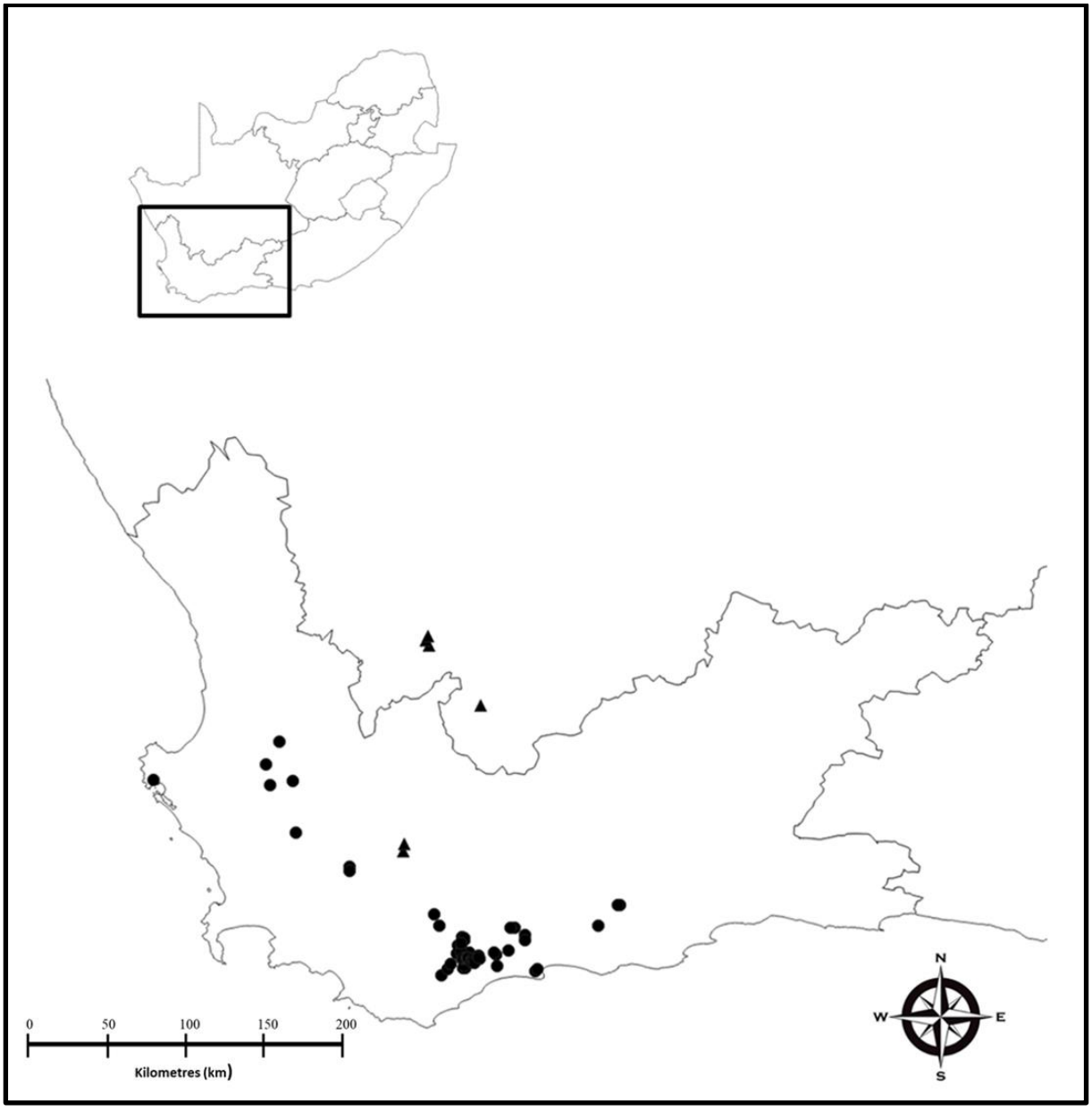

Figure 5: Map of the distribution of subgen. *Polhillia* (circles) and subgen. *Roseopolhillia* (triangles).

5.3. *Polhillia* subgen. *Roseopolhillia* B.du Preez, *subgen. nov*.

5.3.1. Taxonomy

Type species: *Polhillia involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte, Kew Bulletin 44: 3 (1989). **Type:** Thunberg s.n. (UPS-THUNB 17575!, lectotype, chosen by Stirton, 1986b).

Woody shrub, erect, sprouting from rootstock after fire; stipules 2, deltoid-oblong, glabrous with three prominent veins, connate around base, sheathing stem, often persistent; leaves digitately trifoliolate, shortly petiolate, glabrous or rarely sparsely hairy, conduplicate; inflorescence terminal, flowers in clusters of 2–5, subsessile, surrounded by enlarged terminal stipules; flowers rose pink or rarely white, 10–12 mm long; calyx with trifid ventral lip, sparsely pilose, green, often flushed maroon; standard petal broadly elliptic, apex emarginate, pubescent only along abaxial midrib; wing petals broadly oblong with lamellate sculpturing along most part of dorsal half; keel petals lunate, pilose along ventral fore edge; stamens 10, monodelphous with single dorsal slit, filaments fused for most of their length, anthers alternating between long and short; pistil sericeous, variable in length, ovules 7, style sub-erect, stigma regular, capitate; pod dehiscent, pale cream-brown, linear, laterally compressed between seeds, sericeous; seeds ovate-squared, smooth, dark-brown to mottled pale-orange.

5.3.2. Species included:

1. ***Polhillia involucrata*** (Thunb.) B.-E.van Wyk & A.L.Schutte, Kew Bulletin 44: 3 (1989). *Argyrolobium involucratum* (Thunb.) Harv. Fl. Cap. 2: 75 (1862). *Genista involucrata* (Thunb.) Briq. Étude Cytises Alpes Mar. 119 (1894). *Melolobium involucratum* (Thunb.) C.H.Stirt. S. Afr. J. Bot. 52: 4 (1986). *Psoralea involucrata* Thunb. Prodr. 136 (1800); Fl. Cap. 607 (1823). Type—Thunberg s.n. (UPS-THUNB 17575, lectotype, chosen by Stirton, 1986b).

5.3.3. Etymology

The subgeneric epithet *Roseopolhillia* was chosen in reference to the distinct and diagnostic rose pink coloured flowers seen only in this one species in the genus *Polhillia*.

5.3.4. Diagnostic characters

The single species in *Polhillia* subgen. *Roseopolhillia* is distinguished from species in *Polhillia* subgen. *Polhillia* by having pink or rarely white (*versus* yellow) flowers, pods

dehiscent (*versus* pods indehiscent), leaves mostly glabrous and stipules pubescent only on adaxial surface and glabrous on abaxial surface (*versus* leaves and stipules pubescent).

5.3.5. Distribution

This subgenus (species) is distributed through high altitude inland Renosterveld of the Roggeveld Escarpment and Touwsriver areas (Figure 5). All known populations occur at altitudes between 1000–1500 m. This subgenus is for the most part endemic to the Extra Cape Region (ECR) of the GCFR *sensu* Snijman (2013), but just reaches into the CCR *sensu* Manning and Goldblatt (2012) south of Touwsriver.

6. CONCLUSION

This study provides conclusive evidence for *Polhillia* to be a monophyletic genus. Morphological evidence, backed by results from previous studies on SA Genisteae, show that *Polhillia* is morphologically distinguishable from other genera in South Africa. This is also the first study to use molecular phylogenetic analysis to investigate the monophyly of *Polhillia*, and although it generally produced poor internal resolution, the monophyly of the genus was strongly supported. Although *Polhillia involucrata* differs consistently from other species in the genus in having pink flowers and dehiscent fruit, collective morphological characters and molecular phylogenetic evidence propose its retention within *Polhillia*. Its unique traits, combined with its predominantly ECR distribution, support its demarcation here as the new monotypic subgenus *Polhillia* subgen. *Roseopolhillia*. The remaining 10 species in the genus are strongly supported as a clade in the molecular phylogenetic reconstructions, which is supported by their shared posession of yellow flowers and indehiscent fruit, and are thus recognized in subgen. *Polhillia*.

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Declaration by the candidate (for chapter prepared for South African Journal of Botany)

With regards to Chapter 3 (**THE GENUS *POLHILLIA* C.H.STIRT. (GENISTEAE: FABACEAE): A MONOGRAPH**), the nature and scope of my contribution were as follows:

Nature of contribution	Extent of contribution (%)
Data collection, analysis, interpretation and manuscript preparation	80

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Declaration by co-authors:

The undersigned hereby confirm that

2. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 3.
3. No other authors contributed to Chapter 3 than those specified above.
4. There are no conflicts of interest relevant to Chapter 3 of this dissertation.

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CHAPTER 3

THE GENUS *POLHILLIA* C.H.STIRT. (GENISTEAE: FABACEAE): A MONOGRAPH

ABSTRACT

Polhillia is a small and poorly known genus of legumes endemic to the Greater Cape Floristic Region (GCFR) of South Africa. The genus consisted of eight known species at the start of this study, all of which are threatened with extinction. Species boundaries were, however, unclear and many Renosterveld fragments in the Overberg in particular required further exploration in pursuit of the various *Polhillia* species. Through widespread field sampling and analysis of herbarium specimens, we present an updated taxonomy of the genus *Polhillia* that includes the description of 4 new species (*P. groenewaldii* B.du Preez, *P. fortunata* B.du Preez, *P. stirtoniana* B.du Preez, *P. xairuensis* B.du Preez), and the reduction of a previously recognized species (*Polhillia canescens* C.H.Stirt.) into synonymy under *Polhillia connata* (Harv.) C.H.Stirt. Molecular phylogenetic trees within *Polhillia* were largely unresolved and could not be used to support morphological species concepts. Pollen and leaf anatomical studies did not provide substantial taxonomically informative characters either, although some interspecific differences were noted. Red data list assessments are provided for all species to highlight their conservation importance.

1. INTRODUCTION

Fabaceae is globally a diverse family of over 19 500 species distributed across all continents except Antarctica (Lewis *et al*., 2005; LPWG, 2013, 2017). Three subfamilies have traditionally been recognised based primarily on morphological characters, namely Papilionoideae, Mimosoideae and Caesalpinioideae (Lewis *et al*., 2005; Tucker, 2003). A new classification has, however, recently been proposed suggesting that six subfamilies are recognised, and that Mimosoideae be included within the Caesalpinioideae (LPWG, 2017). The Papilionoideae is the most diverse subfamily represented by over 14 000 species from more than 503 genera (Cardoso *et al*., 2013; Lewis *et al*., 2005; LPWG, 2017). The Papilionoideae are characterized by the papilionoid flower, consisting of a standard petal along with two wing and two keel petals, found in the majority of species in this subfamily (Cardoso *et al*., 2013; Tucker, 2003). The recognition of 28 tribes in the Papilionoideae is based on both morphological and molecular phylogenetic data (Cardoso *et al*., 2013; Käss and Wink, 1997). The tribe Genisteae is characterized by the presence of a bilabiate calyx, with two teeth dorsally and three teeth ventrally, also referred to as a trifid lip (Lewis *et al*., 2005; Polhill, 1976). Genisteae is represented by *ca*. 618 species in 25 genera distributed through the temperate regions of the world (Lewis *et al*., 2005; Schrire *et al*., 2005). The most diverse genus in the tribe is *Lupinus* L. that consists of *ca*. 275 species and is well-known for its recent and rapid species radiation in the Andes Mountains (Cardoso *et al*., 2013; Hughes and Eastwood, 2006).

Fabaceae is the second most diverse family in the Greater Cape Floristic Region (GCFR), only outnumbered by the Asteraceae (Manning and Goldblatt, 2012). With over 764 recognized species, legume diversity is often high in many vegetation types in the GCFR (Manning and Goldblatt, 2012). The Tribe Crotalarieae is the most diverse group and includes the mega diverse genus *Aspalathus* L., which consists of over 280 species (Cardoso *et al*., 2013; Dahlgren, 1988; Trytsman *et al*., 2011). Other large genera in the GCFR include *Psoralea* L. (ca. 70 species) and *Otholobium* C.H.Stirt. (*ca*. 50 species; Psoraleeae), and *Indigofera* L. (*ca*. 90 species; Indigofereae) (Cardoso *et al*., 2013; Manning and Goldblatt, 2012; Trytsman *et al*., 2011). Within the GCFR, the tribe Genisteae is under-represented in terms of generic or species diversity (4 genera and *ca*. 40 species), which is in stark contrast to overall legume diversity patterns in GCFR (Manning and Goldblatt, 2012; Snijman, 2013).

Polhillia C.H.Stirt. is a small genus endemic to the GCFR and is a member of the Genisteae based on morphological and molecular phylogenetic data (Cardoso *et al*., 2013; Manning and Goldblatt, 2012; Snijman, 2013; Stirton, 1986a; Swanepoel *et al*., 2015; van Wyk and Schutte, 1995). The genus at present comprises eight recognized species (Table 1), seven of which occur in the Core Cape Subregion (CCR) of the GCFR (Curtis *et al*., 2013; Manning and Goldblatt, 2012), while *Polhillia involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte occurs on the Roggeveld Escarpment of the Extra Cape Subregion (ECR) in the Northern Cape Province (Snijman, 2013).

Table 1: Summary of *Polhillia* currently recognized species along with synonyms.

Species	**Publication**	**Synonyms**
Polhillia brevicalyx (C.H.Stirt.) B.-E.van Wyk & A.L.Schutte	Kew Bulletin 44: 3 (1989)	• *Argyrolobium brevicalyx* (C.H.Stirt.)
Polhillia canescens C.H.Stirt.	S. Afr. J. Bot. 52: 2 (1986)	
Polhillia connata (Harv.) C.H.Stirt.	S. Afr. J. Bot. 52: 2 (1986)	• *Argyrolobium connatum* Harv. • *Genista connata* (Harv.) Briq.
Polhillia curtisiae C.H.Stirt. & Muasya	S Afr. J. Bot. 87 (2013)	
Polhillia ignota Boatwr.	S. Afr. J. Bot. 76 (2010)	
Polhillia involucrata (Thunb.) B.-E.van Wyk & A.L.Schutte	Kew Bulletin 44: 3 (1989)	• *Argyrolobium involucratum* (Thunb.) Harv. • *Genista involucrata* (Thunb.) Briq. • *Melolobium involucratum* (Thunb.) C.H.Stirt. • *Psoralea involucrata* Thunb.
Polhillia obsoleta (Harv.) B.-E.van Wyk	Bothalia 22: 1 (1992)	• *Polhillia waltersii* (C.H.Stirt.) C.H.Stirt.
Polhillia pallens C.H.Stirt.	S. Afr. J. Bot. 52: 2 (1986)	

Polhillia has a unique suite of morphological characters such as connate stipules, which are adnate to the petiole and sheath the stem, along with the presence of pseudo-peduncles, much reduced or absent bracts and bracteoles that separates it from other South African Genisteae (Stirton, 1986a; van Wyk and Schutte, 1989). Species level circumscription has focussed primarily on macro-morphological characters such leaf and flower morphology (Boatwright,

2010; Curtis *et al*., 2013; Stirton, 1984, 1986a). All *Polhillia* species have conduplicate trifoliolate leaves that vary in dimension and pubescence, which provide useful interspecific taxonomic characters (Boatwright, 2010; Curtis *et al*., 2013; Stirton, 1984, 1986a; van Wyk and Schutte, 1989). Floral morphology typically provides important characters for species-level taxonomy (Ornduff, 1968), although variation between closely related papilionoid legumes species can be cryptic (Aïnouche *et al*., 2004; Stirton, 1986a). Flower colour variation within legume genera can often be invariable (Bello *et al*., 2017; Boatwright *et al*., 2009; Campbell and van Wyk, 2001; Dahlgren, 1988; Dludlu *et al*., 2015; Edwards, 1994; Kies, 1951; Moteetee and van Wyk, 2006; Schutte-Vlok and van Wyk, 2011). *Polhillia* follows this trend as seven of the eight species recognised, have yellow flowers and only *P. involucrata* has pink flowers (Curtis *et al*., 2013; Manning and Goldblatt, 2012; Snijman, 2013; Stirton, 1986a; van der Merwe, 2010). Petal dimension, pubescence and sculpturing are, however, usually important characters when assessing species-level differences in legumes (Bello *et al*., 2017; Campbell and van Wyk, 2001; Dahlgren, 1988; Edwards, 1994). Calyx morphology can be more useful for species differentiation than the corolla among papilionoid legumes (Bello *et al*., 2017; Dahlgren, 1988; Schutte-Vlok and van Wyk, 2011). The calyx dimensions, pubescence and teeth morphology provide characters useful for distinguishing between species, including the differentiation of some *Polhillia* species (Boatwright, 2010; Curtis *et al*., 2013; Stirton, 1986a, 1984). *Polhillia* flowers are borne on pseudo-peduncles or occur in terminal subsessile clusters varying from 1–5 flowers , which is considered useful for species-level taxonomy (Boatwright, 2010; Curtis *et al*., 2013; Stirton, 1986a, 1984; van Wyk and Schutte, 1989). The length of the pseudo-peduncles along with the number of flowers were highlighted as important characters when differentiating between *P. ignota* and *P. obsoleta* (Boatwright, 2010). All *Polhillia* species have indehiscent pods, with the exception of *P. involucrata*, which are either plicate or laterally compressed between seeds (Boatwright, 2010; Curtis *et al*., 2013; du Preez *et al*., 2019 – Chapter 2; Stirton, 1986a, 1984; van Wyk and Schutte, 1989). Seed morphology is generally poorly studied in GCFR legumes, including in *Polhillia* as seed morphology of only two species has been described (Stirton, 1986a). When revising *Melolobium* Eckl. & Zeyh., Moteetee and van Wyk (2006) found some variation in seed size and mottling patterns, although testa colour was not useful as a taxonomic character. Legume seed morphology is often taxonomically informative in tree species (Gunn, 1981; Kirkbride *et al*., 2003; Rodrigues *et al*., 2014; Smit, 2012), and should thus be explored further among all *Polhillia* species and other GCFR legumes.

Palynology has been widely used in systematic studies (Banks and Rundall, 2016; Dreyer, 1996; Zhao *et al*., 2016). Interspecific pollen morphological variation may be low due to strong selection against change in pollen morphology (Dehgan and Dehgan, 1988; Muller, 1979; Solomon, 1983), but many studies have found substantial variation between species (*e.g*., Pardo *et al*., 2000; Umdale *et al*., 2017; Zhao *et al*., 2016). Substantial interspecific pollen morphological variation has been found in many studies involving legumes (González-Vázquez *et al*., 2017; Gunes and Aytug, 2010; Pardo *et al*., 2000; Umdale *et al*., 2017; Zhao *et al*., 2016). A unique pollen type among legumes with asymmetrical pollen grains containing one ecto-aperture and only two endo-apertures was discovered in *Duparquetia orchidacea* Baill. (Banks *et al*., 2006). Legumes predominantly have tricolpate pollen, although shape and surface texture differ greatly between genera and species (Banks *et al*., 2006; Pardo *et al*., 2000; Umdale *et al*., 2017; Zhao *et al*., 2016). Genisteae pollen morphology has been suggested to be relatively homogeneous (Ferguson and Skvarla, 1981), although studies on some Mediterranean Genisteae have shown some interspecific variation (Cubas and Pardo, 1992; Ferrauto *et al*., 2015; Pardo *et al*., 2000; Rizzi Longo *et al*., 2006; Rizzi Longo & Feoli Chiapella, 2007, 2009). It is therefore worthwhile investigating pollen morphology in *Polhillia* as it is a novel study and may provide taxonomically informative characters.

Leaf anatomy has proven to be of substantial systematic significance among angiosperms (Jooste *et al*., 2016; Lauterbach *et al*., 2016; Matias *et al*., 2007; Moteetee *et al*., 2002). Leaf anatomy has proven particularly useful, and offers systematic significant traits such as mesophyll structure, shape and position of stomata (Jooste *et al*., 2016) and arrangement of vascular bundles. Lauterbach *et al*. (2016) used three leaf anatomical traits in Zygophyllaceae to show the evolution of traits beneficial to arid environments, comparing both phylogenetic and habitat perspectives. Interestingly, this study found multiple shifts in leaf traits, with leaf traits distributed unevenly across the phylogeny (Lauterbach *et al*., 2016). While leaf lamina anatomy has not been widely studied in legumes, petiole anatomy has been studied fairly widely in legumes (e.g., Al-Joboury, 2016; Amirabadizadeh *et al*., 2015; Leme and Scremin-Dias, 2014; Potgieter and Wessels, 1998; van Wyk and Schutte, 1989). Petiole morphology was used by van Wyk and Schutte (1989) as evidence to include *Polhillia involucrata* and *Polhillia brevicalyx* in the genus, but did not investigate lamina anatomy. Investigating leaf lamina anatomy has not been done for any *Polhillia* species, and may provide characters useful for species-level taxonomy.

Although morphology is useful for separating species with clear species boundaries, molecular phylogenetic data may be useful as support for morphological species concepts. *Polhillia* species have been included in wide scale molecular phylogenetic studies (Boatwright *et al*., 2008; Swanepoel *et al*., 2015), but no studies have investigated the interspecific relationships between *Polhillia* species until du Preez *et al*. (2019 – Chapter 2). Although poor internal resolution was found for individual markers, our combined nuclear and plastid tree provided relatively good species-level resolution. We aim to use this tree to compare with our morphological species concepts.

There has been much confusion over the identity of several of the Overberg species due to similar morphological features, especially when looking at old dried specimens. Recent collections made through field studies in the area by the Overberg Renosterveld Conservation Trust (ORCT), along with botanists such as Profs Stirton and Muasya, have brought to light morphological variation among *Polhillia* populations in this region. A new taxon from east of Swellendam was speculated to be a subspecies of *Polhillia pallens s.l.* (Stirton, *pers. comm.*). The mystery around *Polhillia connata* remains unresolved and needs attention, as the species may be extinct. Many areas of Renosterveld in the GCFR remain under-explored and may hold new localities of these rare species or even species new to science. The main aim of this monograph was thus to resolve the mysteries surrounding the taxonomy of the genus *Polhillia* following a multi-disciplinary approach using morphological, palynological, anatomical and molecular phylogenetic data. To ensure best conservation practice of a threatened genus, fieldwork was mostly centred in very threatened Renosterveld vegetation types. We further also aimed to reassess the conservation status of each species recognised in this monograph following the IUCN categories and criteria (IUCN, 2012).

2. HISTORICAL REVIEW

The genus *Polhillia* was described by Prof. C.H. Stirton in 1986 after he split *Lebeckia waltersii* C.H.Stirt. (subgenus *Plecolobium*) from the broadly circumscribed genus *Lebeckia* Thunb. (Stirton, 1986a). *Polhillia waltersii* (C.H.Stirt.) C.H.Stirt. was thus circumscribed as the type of the genus (Stirton, 1986a). At the time of describing the genus, Prof. Stirton included *Argyrolobium connatum* Harv. in the genus and described 2 new species, *Polhillia canescens* C.H.Stirt. and *Polhillia pallens* C.H.Stirt. (Stirton, 1986a). He further made reference to a single specimen from the West Coast, which he referred to as *Polhillia sp. A* (Stirton, 1986a), and viewed it as a potentially new species (Figure 1). An odd species in the

overall context of South African Genisteae, first described as *Psoralea involucrata* Thunb., was not included in *Polhillia* by Stirton (1986a), but rather circumscribed as *Melolobium involucratum* (Thunb.) C.H.Stirt. (Stirton, 1986b). This species was, however, later included by van Wyk and Schutte (1989) when broadening the concept of the genus *Polhillia* to include this strange species, *Polhillia involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte based on morphological and anatomical characters, chromosome numbers and alkaloid compositions (van Wyk *et al*., 1988). *Argyrolobium brevicalyx* C.H.Stirt. was described in 1984, but also only circumscribed as *Polhillia brevicalyx* (C.H.Stirt) B.-E.van Wyk & A.L.Schutte in 1989 (Stirton, 1984; van Wyk and Schutte, 1989). *Polhillia waltersii* was later synonymised by van Wyk (1992) after an old specimen of Thunberg that was described as *Argyrolobium obsoletum* Harv. was located and circumscribed as *Polhillia obsoleta* (Harv.) B.-E.van Wyk. *Polhillia sp. A* was finally described as *Polhillia ignota* Boatwr. in 2010 and one further new species, *Polhillia curtisiae* C.H.Stirt. & Muasya, was described in 2013 (Boatwright, 2010; Curtis *et al*., 2013).

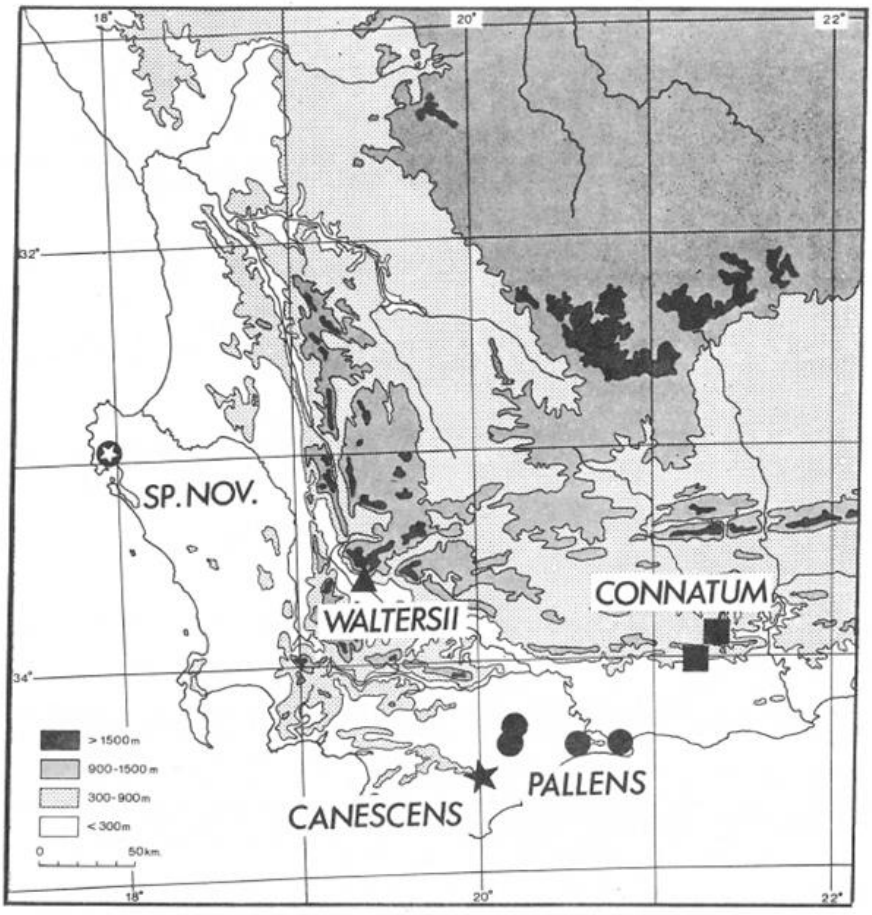

Figure 1: Map from Stirton (1986a) showing the distribution of species recognized in *Polhillia* in 1986.

Polhillia species have been sporadically collected since the beginning of botanical exploration by European settlers in the Cape. Early in the 19th century, Carl Thunberg collected the type specimens of *Polhillia obsoleta* and *Polhillia involucrata*, while Rev. George Thom collected the type specimen of *Polhillia connata* (Harv.) C.H.Stirt. (Stirton, 1986a; van Wyk, 1992). Two specimens of *Polhillia ignota* were collected early in the 20th century. John Muir collected *Polhillia connata* at three localities in the 1920's, while type specimens of *Polhillia pallens* C.H.Stirt., *Polhillia brevicalyx* (C.H.Stirt.) B.-E.van Wyk & A.L.Schutte and *Polhillia canescens* C.H.Stirt. were first collected in the 1960's, 1970's, and 1980's (Stirton, 1986a, 1984). The slow and sporadic historical discovery and collection of members of *Polhillia* raises questions about the natural abundance of this genus. Are *Polhillia* species naturally rare or has habitat fragmentation due to agriculture caused the rare and patchy distribution of current day species? Most species grow on clay-rich soils, which has historically been favoured for growing wheat and other crops. The highest concentration of species occurs in the Overberg region, of which many parts were not well-explored until at least the 1960's. Many of the species in this area were collected by Chris Burger and Bruce Bayer in the 1970's and 1980's, where after collections became scarce until the early 2000's when research into Overberg Renosterveld became more focussed (Odette Curtis, *pers. com.*). Results of this renewed focus highlighted the incredible botanical diversity remaining within the small Renosterveld fragments of this region.

Many *Polhillia* species and collections are shrouded in mystery. The first mystery involves the type collection of *Polhillia connata* by Rev George Thom, who provided no locality details or date of collection. It is thought that the specimen may have been collected in the Caledon area, but it has never been collected there since. The collections by John Muir were tentatively named as *Polhillia connata* by Stirton (1986a), although these were collected in the Riversdale area and have subsequently also not been relocated. A collection was made in 2008 of what was thought to be *Polhillia connata* on the farm Haarwegskloof in the southern Overberg, but this was later described as *Polhillia curtisiae* (Curtis *et al*., 2013). *Polhillia ignota* or previously known as "*Polhillia sp. A*" (Stirton, 1986a), was presumed extinct after not being relocated for roughly 90 years, until we rediscovered a small population north of Piketberg in 2016 (Ebrahim *et al*., 2016).

3. MATERIALS & METHODS

3.1. Morphology

3.1.1. Material collection

Voucher specimens of all *Polhillia* species were collected at as many sites as possible during extensive fieldwork. Fieldwork was conducted throughout the study period of 2017 and 2018 to maximise the number of *Polhillia* populations sampled. Field trips were directly intended to locate new and known populations, throughout the known and suspected distribution range of this genus. At each collection site, herbarium voucher specimens were made, along with both fresh, 50% Formalin-Acetic-Acid (FAA) stored and silica dried material used for morphological, anatomical, palynological and molecular analyses. Seed was collected from ripe pods and placed in a freezer at -20°C for 48 hours to kill any insects, before being stored in a cool, dry place until use. Specimens were deposited in both the Stellenbosch University herbarium (STEU) and the Bolus herbarium (BOL) at the University of Cape Town. Additional material was collected of type collections to be distributed to other herbaria including NBG, PRE, PRU, SCHG, K and MO. Herbaria abbreviations are given according to Thiers (2017).

3.1.2. Morphological examination

Analysis of morphological characters started in the field, but most comparative analyses were based on herbarium specimens and material stored in FAA. Field observations were made on characters including plant height, width, and growth form as well as fire survival strategy. Morphological analysis of species was based on both old and new specimens (own collections as well as material from BOL, K, NBG, PRE), including all available type specimens. Several type specimens were also viewed online through JSTOR Plants (https://plants.jstor.org). Macro-measurements were taken using callipers, while micro-measurements were made using a Leica M125 stereomicroscope with an attached Leica MC170 periscope camera, using LAS v4.9 software (Leica Microsystems, 2018a). At least 10 measurements were made per trait per specimen and at least three (but mostly up to 10) specimens were studied per species to account for variation.

3.1.3. Palynology

Pollen was collected from virgin flowers from herbarium specimens collected during the first fieldwork season (2017). Pollen-laden anthers were dissected from the flower using ethanol-sterilized forceps and rubbed onto carbon tape stuck onto aluminium stubs. Stubs were sputter-coated with gold-palladium for 3 minutes following standard protocols (Brunk *et al*.,

1981), whereafter a Zeiss Merlin FEG Scanning Electron Microscope (SEM) was used to study pollen morphology. Photographs of polar and equatorial views of pollen grains were taken at 3000x magnification, while close-ups of the tectum were taken at 10 000x magnification.

3.1.4. Leaf Anatomy

Fresh leaves were fixed in 50% Formalin-Acetic-Acid (FAA). FAA fixed material was then dehydrated in an ethanol series and infiltrated with, and ultimately imbedded in paraffin wax (Johansen, 1940). Laminar cross sections ranging in thickness between 10-30 µm were made using a Leica Wetzlar rotary microtome. Sections were mounted onto microscope slides and stained using the Safranin-Alcian-blue method (Johansen, 1940). Permanent slides were made using long cover slips and DPX glue. The sections were studied using a Leica DM500 light microscope, while a Leica ICC50 W camera was used to capture images using LAS EZ v3.3.0 software (Leica Microsystems, 2018b).

3.2. Molecular phylogenetic analysis

CTAB extraction protocols followed and sample information are detailed in du Preez *et al*. (2019 – Chapter 2). Polymerase Chain Reactions (PCR) were run using nuclear Internal Transcribed Spacer (ITS) and plastid (trnS-G, psbA-trnH) markers. For ITS, the universal AB101 and AB102 primer pair was used (Sun *et al*., 1994) and the reaction was run using the following protocol: initial denaturation at 94°C for 5 min; 35 cycles of 94˚C for 60 s, 61˚C for 60 s and 72˚C for 90 s, respectively; final extension for 6 minutes at 72˚C. For the trnS-G region, we used the trnS(GCU) and trnG(UUG) primer pair (Hamilton, 1999) and PCR conditions were set as follows: initial denaturation for 5 min at 96°C; 35 cycles of 96°C for 45 s, 52°C for 1 min, 72°C for 1 min; final extension at 72°C for 7 min. For the psbA-trnH region we used the primer pair psbA and trnH(GUG) primer pair (Hamilton, 1999) and PCR conditions were set as follows: 95 for 3 min; 35 cycles of 95 for 30 s, 55 for 1 min, 72 for 90 s; 72 for 4 min (Sang *et al*., 1997). Direct sequencing was done at the Stellenbosch Central Analytical Facility (CAF). Sequences were edited using Chromas2 v2.3 (Technelysium Pty Ltd, 2004), and aligned and analysed using BioEdit v7.2.5 (Ibis Biosciences, 2013). Phylogenetic analyses were done through Bayesian Inference (BI) using MrBayes v3.2 (Ronquist and Huelsenbeck, 2003) under nst=mixed model of nucleotide substitution and an invgamma parameter to account for among site rate variation, for 10^6 generations and trees were sampled every 2000 generations under otherwise standard settings. All runs reached

stationarity on the posterior as judged by MrBayes internal diagnostics. Phylogenetic consensus trees were viewed and analysed using FigTree v1.4.3 (Rambaut, 2009).

3.3. Phytogeography

Phytogeographic information was obtained during the collection of specimens and population site visits during fieldwork. GPS co-ordinates were obtained using a handheld Garmin GPS.

4. RESULTS

4.1. Taxonomic significance of different disciplines

4.1.1. Morphology

4.1.1.1. Habit

Although all *Polhillia* species sprout after fire, they display a wide array of habits. All species are woody shrubs, but the dimensions, and importantly also the branching patterns, differ between species. *Polhillia stirtoniana* B.du Preez *sp. nov.* (Section 6, number 10) and *P. xairuensis* B.du Preez *sp. nov.* (Section 6, number 8) display dense terminal burst branching to form shrubs with closed canopies, while other species display relatively dense branching but do not have closed canopies. *Polhillia connata* has an erect, lax growth form with sparse branching. All species have erect growth forms, except *P. brevicalyx* which is typically decumbent.

4.1.1.2. Bark

Bark traits were studied with caution, as they varied according to the age of the plants, and the position on the plant. Informative traits included bark colour, texture, presence and abundance of lenticels. Bark colour of *P. ignota* and *P. obsoleta* was golden-yellow fading brown, while bark of all other species was typically grey-brown in colour.

4.1.1.3. Leaves

Leaf morphological traits investigated include physical measurements of the shape and size of leaves, as well as density and type of pubescence. *Polhillia* species were found to display interspecific leaf morphological differences, which remained fairly consistent within species. Even among putatively closely related species, such as those from the Overberg region, leaf morphology proved useful in species identification and circumscription. Useful traits included the size and shape of the leaves, but even more importantly the indumentum of leaves. Seasonal variation in the level of leaflet conduplication was noted, which complicated matters somewhat. Tightly conduplicate leaves observed during the hot, dry summer looked

very similar between species, but when leaves opened up during cool, moist winter months; interspecific differences were much more obvious. Leaf size also varied based on the age of the plant, with younger plants having larger leaves than older individuals of the same species.

4.1.1.4. Inflorescence

The arrangement of *Polhillia* flowers proved to be a useful and reliable taxonomic character between putatively distantly related *Polhillia* species, but less so between closely related species. In the absence of a true peduncle, most species have flowers emerging terminally on branches on short pedicels, while some species are pseudo-pedunculate. For the species without pseudo-peduncles, the main floral arrangement character found to be useful was the number of flowers per branch tip. Putatively closely related species were often reliably separated based on whether flowers occur singly or in terminal pairs, although some variation was seen. Species with 2-5 flowers per branch tip were generally easily separated based on other morphological characters. Pedicel lengths for most species were similar, up to about 3.5 mm long, except for *P. involucrata* (up to 6 mm) and up to 7 mm long in *P. fortunata* B.du Preez *sp. nov.* (Section 6, number 3). Pedicel length was thus used with caution in this study.

4.1.1.5. Calyx

All species share a bilabiate calyx with a trifid ventral lip. Detailed calyx morphology between the various *Polhillia* species varied, but was not very useful when comparing putatively closely related species. Calyx traits that could be used to define species included the total length of the calyx, the tube and teeth length individually, as well as type of indumentum. *Polhillia connata* has narrowly-triangular to ensiform ventral calyx teeth that converge to each other, while all other species have triangular ventral calyx teeth that rarely converge. All species had densely pubescent calyces, except *P. involucrata* that has a sparsely pilose calyx. The inner tips of the calyx teeth were pubescent in all species and therefore were not taxonomically informative.

4.1.1.6. Corolla

Corolla morphology displayed little consistency in characters, with subtle differences found even intra-specifically. Standard petal shape was ovate to broadly ovate in all species, except *P. involucrata* that has a broadly elliptical standard. Pubescence patterns on the back of the standard petals varied even within species and no definite patterns could be established. Wing petals were the most taxonomically informative floral parts, and consistently differed in shape and sculpturing between species. Although these traits also showed intra-specific variation, they remained consistent enough to be taxonomically informative at the species

level. Although keel petals also showed both inter- and intra-specific variation in shape and pubescence, keel morphology was also taxonomically useful to some degree.

4.1.1.7. Androecium

Androecium morphology displayed subtle, yet useful, taxonomic characters. The most informative character was the arrangement of the anthers around the style. Anthers either remained closely packed around the style or spread away from the style. In species with spreading anthers, the spread was both neat and remained equidistant from the style or the spread was disorderly and random. All *Polhillia* species have 10 anthers, 5 are basifixed and the other 5 are dorsifixed (Figure 2). The dorsifixed anthers are substantially shorter than the basifixed anthers and are ovate in all species, except *P. xairuensis* B.du Preez *sp. nov.* (Section 6, number 8) that has elliptical short anthers. Basifixed anthers comprise of 4 long anthers and one carinal anther that is elliptical, lanceolate or oblong in shape. Carinal anthers were typically intermediate in length between long and short anthers, except in *P. connata*, *P. curtisiae*, *P. obsoleta* and *P. involucrata* in which they were as long as the long anthers. Anther length between closely related species was similar and thus not a useful taxonomic character. *Polhillia connata* has substantially larger anthers than all other *Polhillia* species with long anthers reaching up to 2.5 mm in length and short anthers up to 1.3 mm.

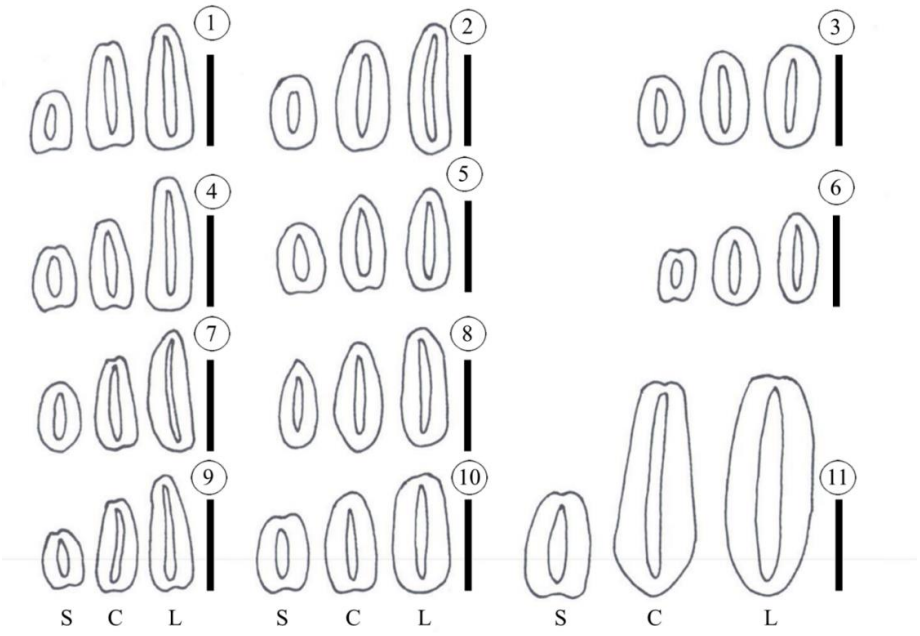

Figure 2: Line drawings of side view of short (S), carinal (C), and long (L) anthers of all *Polhillia* species. 1 – *P. obsoleta*; *P. stirtoniana*; 3 – *P. involucrata*; 4 – *P. ignota*; 5 – *P. curtisiae*; 6 – *P. brevicalyx*; 7 – *P. fortunata*; 8 – *P. xairuensis*; 9 – *P. groenewaldii*; 10 – *P. pallens*; 11 – *P. connata*. Scale bars: 1 mm. Illustration by B. du Preez.

4.1.1.8. Gynoecium

Gynoecium morphology of *Polhillia* species is similar, with ovaries of all species densely covered in long sericeous hairs, except for *P. brevicalyx* that has a villous ovary. Ovary length is variable due to both inter- and intra-specific variation in flower length. Ovule number remained consistent for the most part between putatively closely related species, with most Overberg species having 9 ovules, except *P. stirtoniana* B.du Preez *sp. nov.* (Section 6, number 10) that has 9–11 ovules. *Polhillia ignota*, *P. obsoleta*, *P. fortunata* B.du Preez *sp. nov.* (Section 6, number 3) and *P. groenewaldii* B.du Preez *sp. nov.* (Section 6, number 5), *P. brevicalyx* and *P. involucrata* only have 7 ovules. The angle of incline of the style and the height of the stigma proved to be taxonomically important and were particularly useful to separate some of the Overberg species from one another (especially *P. curtisiae*, *P. pallens* and *P. xairuensis*).

4.1.1.9. Fruit

All *Polhillia* species have indehiscent pods, except *P. involucrata*, which has a dehiscent pod. Five species, including *P. ignota*, *P. obsoleta*, *P. fortunata* B.du Preez *sp. nov.* (Section 6, number 3), *P. groenewaldii* B.du Preez *sp. nov.* (Section 6, number 5) and *P. stirtoniana* B.du Preez *sp. nov.* (Section 6, number 10) have plicate pods, while all other species have non-plicate pods. Pods of species with non-plicate pods were similar in shape and size. Pubescence was found to be somewhat useful, as *P. curtisiae* has velutinous pods, *P. brevicalyx* villous pods and all other species have sericeous pods.

4.1.1.10. Seed

Seed morphology in *Polhillia* provided very little variation and was not useful as a taxonomic character (Figure 3). The only species with somewhat different seeds was *P. involucrata* that displayed consistent mottling on the testa and seeds were ovate-squared in shape. Seeds of all other species were ovate-reniform in shape. Occasional mottling was observed on seeds of *P. connata* and *P. pallens*, while other species had no mottling on seed testas. Seed colour varied from lemon yellow to olive green to dark brown-black, but intra-specific variation in colour was observed in most species, to the extent that testa colour was not taxonomically informative.

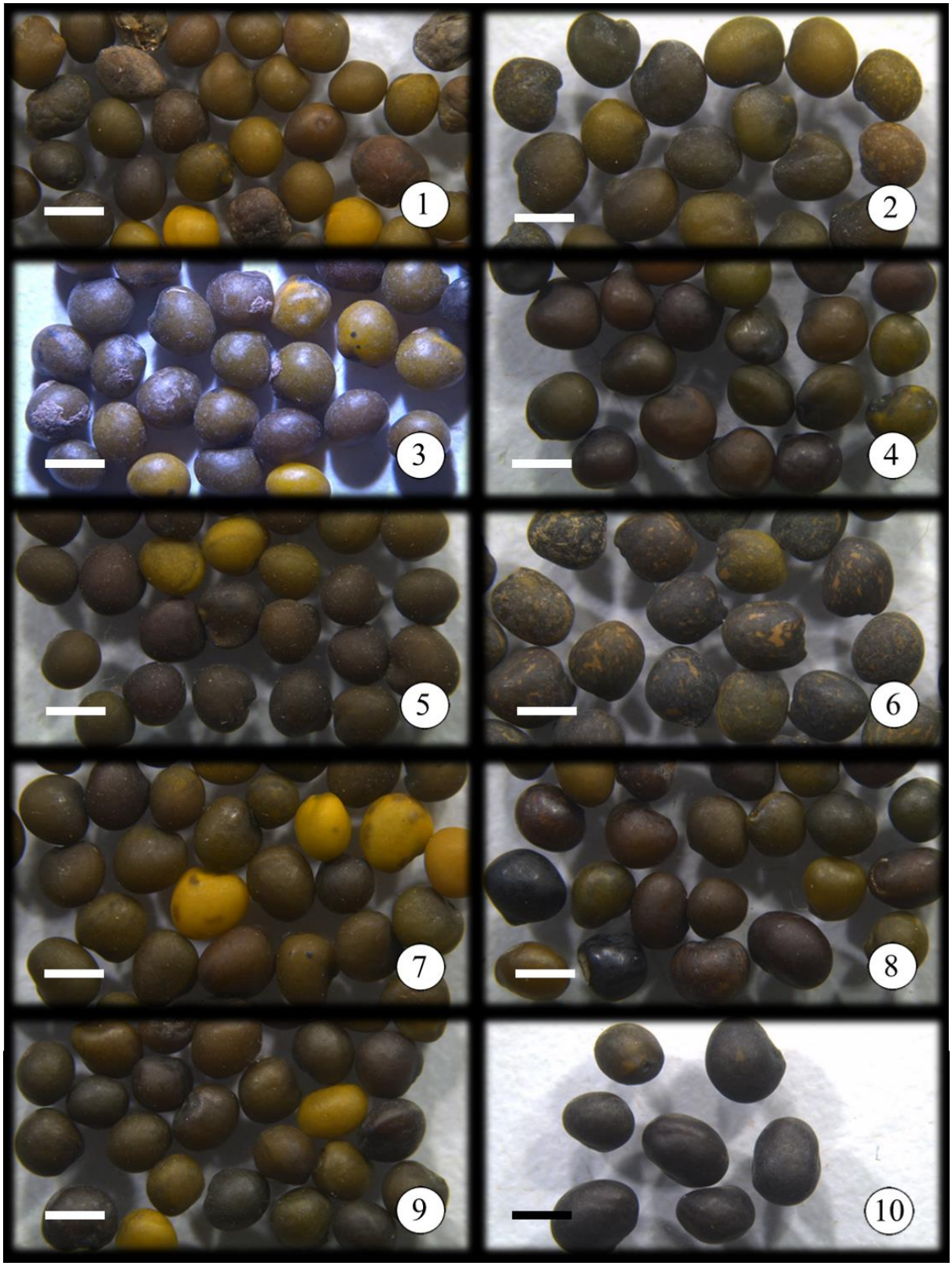

Figure 3: Seed of various *Polhillia* species. 1 – *P. brevicalyx*; 2 – *P. connata*; 3 – *P. groenewaldii*; 4 – *P. curtisiae*; 5 – *P. ignota*; 6 – *P. involucrata*; 7 – *P. obsoleta*; 8 – *P. pallens*; 9 – *P. stirtoniana*; 10 – *P. xairuensis*. Scale bars: 2 mm.

4.1.2. Palynology

Polhillia species showed limited palynological variation. All species have tricolpate pollen that is oblong to ovate in equatorial view and rounded-triangular in polar view (Figure 4). Both pollen grain size and shape were variable at the infra-specific level, rendering these traits taxonomically uninformative.

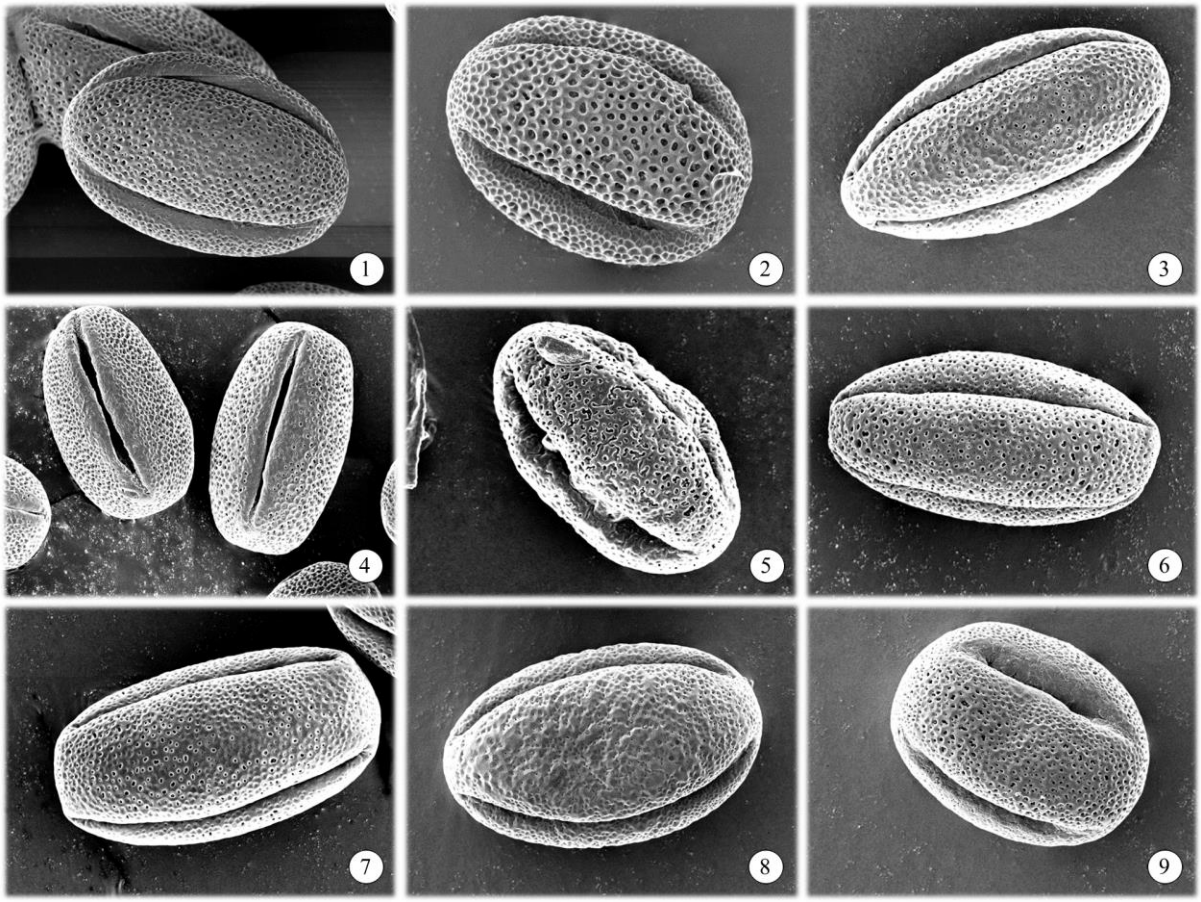

Figure 4: SEM micrographs of the equatorial view of pollen grains of *Polhillia* species. 1 – *P. involucrata*; 2 – *P. connata*; 3 – *P. brevicalyx*; 4 – *P. ignota*; 5 – *P. xairuensis*; 6 – *P. pallens*; 7 – *P. stirtoniana*; 8 – *P. curtisiae*; 9 – *P. obsoleta*. Magnification 3000 x.

Although tectum structure was also fairly similar between species, detailed traits such reticulation patterns of the tectum added some taxonomic insights. Pollen grains are semi-tectate and vary between having a micro-reticulate to coarsely-reticulate tectum, with heterobrochaete lumina and prominent intra-luminary bacules. The coarsely reticulate tectum structure of *P. connata*, with reticulum sizes of 1.0–1.6 µm, set this species apart from all other species with finer reticulums of less than 1.0 µm. The lumina of all species were rounded to oblong, while the lumina of *P. xairuensis* B.du Preez *sp. nov.* (Section 6, number 8) were unique in being elongate to almost channelled (Figure 5).

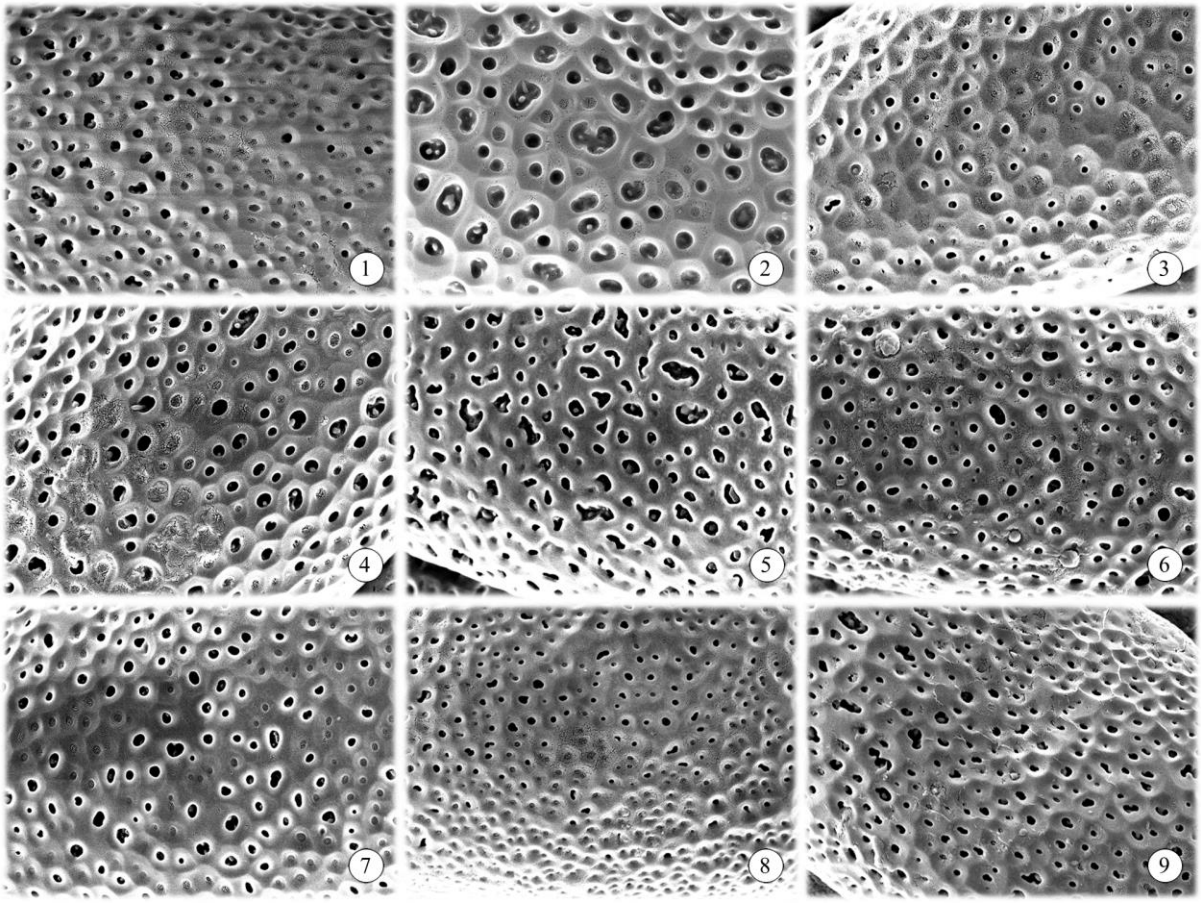

Figure 5: SEM micrographs of the equatorial view of pollen grains of *Polhillia* species. 1 – *P. involucrata*; 2 – *P. connata*; 3 – *P. brevicalyx*; 4 – *P. ignota*; 5 – *P. xairuensis*; 6 – *P. pallens*; 7 – *P. stirtoniana*; 8 – *P. curtisiae*; 9 – *P. obsoleta*. Magnification 10 000 x.

4.1.3. Leaf anatomy

Cross-sections through the leaves of different *Polhillia* species showed surprising diversity in anatomical structure. All Overberg species, except *Polhillia brevicalyx*, displayed very densely packed mesophyll consisting of palisade and spongy tissue and vascular bundles often rich in sclerenchyma (Figure 6.1). Species occurring outside of the Overberg also have densely packed mesophyll consisting of palisade and spongy tissue (except *P. involucrata*), but only *P. ignota* was found to have sclerenchyma within the vascular bundles (Figure 6.2). The four mesophyll arrangement types found among the eleven *Polhillia* species included bifacial, isobilateral (spongy parenchyma centre), isobilateral with palisade only and isobilateral with spongy parenchyma only (Table 2). All species have somewhat conical epidermal cells, except *P. involucrata* and *P. brevicalyx* that have rectangular-oval epidermal cells forming a smooth epidermis. The leaf anatomy of *Polhillia involucrata* and *Polhillia brevicalyx* separated them from all other *Polhillia* species in that they have spacious mesophyll with abundant air spaces, scattered palisade and spongy mesophyll and

rectangular-oval epidermal cells. The adaxial epidermal cells of *P. groenewaldii* B.du Preez *sp. nov.* (Figure 6.2, number 1) are notably smaller than the abaxial epidermal cells, which is unique in the genus as other species have equal sized adaxial and abaxial epidermal cells. All *Polhillia* species have 3-4 vascular bundles per millimetre of leaf width, while *Polhillia involucrata* has 8-9 vascular bundles per millimetre of leaf width.

Table 2: Summary of mesophyll and epidermal differences observed in cross sections through the leaves of the different *Polhillia* species. AD = adaxial; AB = abaxial.

Species	**Mesophyll**	**AD epidermis**	**AB epidermis**
P. brevicalyx	Bifacial	rectangular-oval	rectangular-oval
P. connata	Isobilateral, spongy centre	conical-papillose	conical-papillose
P. curtisiae	Bifacial	conical-papillose	conical-papillose
P. fortunata	Isobilateral, spongy centre	conical-oval	conical-oval
P. groenewaldii	Bifacial	conical-oval	conical-oval
P. ignota	Isobilateral, palisade only	conical-papillose	rectangular-conical
P. involucrata	Isobilateral, spongy centre	rectangular-oval	rectangular-oval
P. obsoleta	Isobilateral, spongy centre	rectangular-conical	rectangular-oblong
P. pallens	Bifacial	conical-papillose	conical-papillose
P. stirtoniana	Bifacial	conical-papillose	conical-papillose
P. xairuensis	Isobilateral, spongy only	conical-papillose	conical-papillose

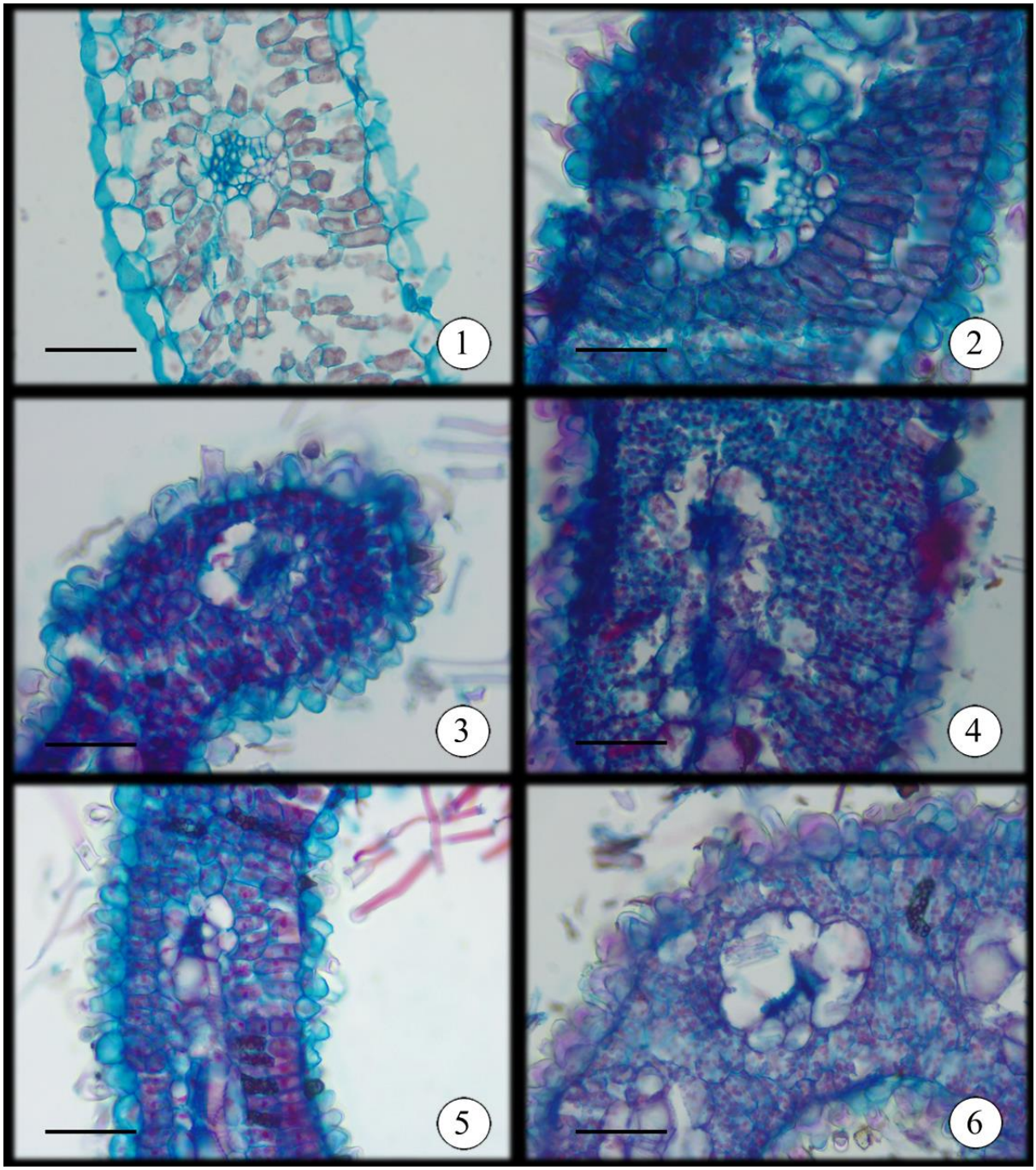

Figure 6.1: Cross-sections through the leaves of *Polhillia* species, with adaxial surfaces on right-hand side of all images. 1 – *P. brevicalyx*; 2 – *P. connata*; 3 – *P. curtisiae*; 4 – *P. pallens*; 5 – *P. stirtoniana*; 6 – *P. xairuensis*. Note the spacious arrangement of mesophyll in *P. brevicalyx*. Furthermore, note the bifacial mesophyll in *P. brevicalyx*, *P. curtisiae*, *P. pallens* and *P. stirtoniana*, and the isobilateral arrangement in *P. connata* with a spongy mesophyll centre and *P. xairuensis* that comprised exclusively of spongy mesophyll. Also note the much thicker leaves of *P. pallens* and *P. connata* compared with the other species. Images taken at magnification of 400x. Scale bars: 50 µm.

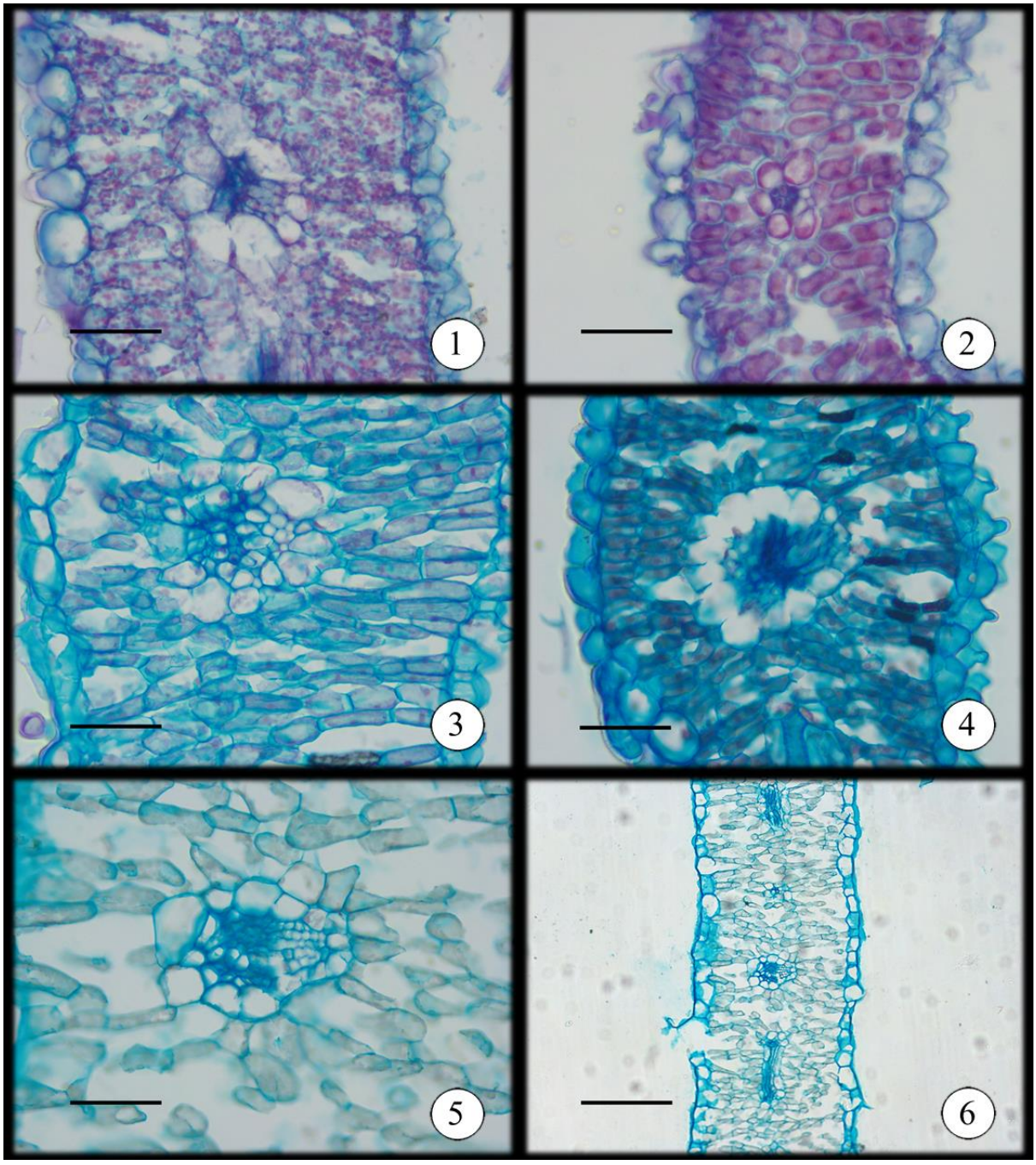

Figure 6.2: Cross-sections through the leaves of *Polhillia* species. 1 – *P. groenewaldii*; 2 – *P. fortunata*; 3 – *P. ignota*; 4 – *P. obsoleta*; 5 & 6 – *P. involucrata*. Note the spacious mesophyll arrangement in *P. involucrata*, similar to *P. brevicalyx* (Figure 6.1). Furthermore, note the bifacial mesophyll of *P. groenewaldii*, while other species have isobilateral mesophyll. Also note that *P. ignota* is the only species with only palisade mesophyll, while other species have palisade mesophyll with spongy centres. *Polhillia involucrata* has the thickest leaves in the genus. Images taken at magnification of 400x (Image 1–5), 100x (Image 6). Scale bars: 50 µm (Image 1–5); 200 µm (Image 6).

4.2. Molecular phylogenetic analysis

Molecular phylogenetic analysis of the genus proved challenging as sequence divergence between *Polhillia* species was minimal at best for most markers attempted. All molecular phylogenetic trees were provided in du Preez *et al*. (2019 – Chapter 2). Here we present a combined nuclear and plastid phylogeny for *Polhillia* (Figure 7) extracted from that paper. Although probability support values are generally poor, most of our morphological species concepts are supported. The clade containing *P. curtisiae*, *P. pallens* and *P. xairuensis* B.du Preez *sp. nov.* (Section 6, number 8) has poor support of only 61% and does not resolve any of these species. The placement of *P. fortunata* B.du Preez *sp. nov.* (Section 6, number 3) in a poorly supported clade with *P. connata* and *P. stirtoniana* B.du Preez *sp. nov.* (Section 6, number 10) is questionable as the species is morphologically similar to *P. ignota*. *Polhillia involucrata* resolves as sister to the rest of the genus with 96% support and was circumscribed into subgen. *Roseopolhillia* B.du Preez (du Preez *et al*., 2019 – Chapter 2).

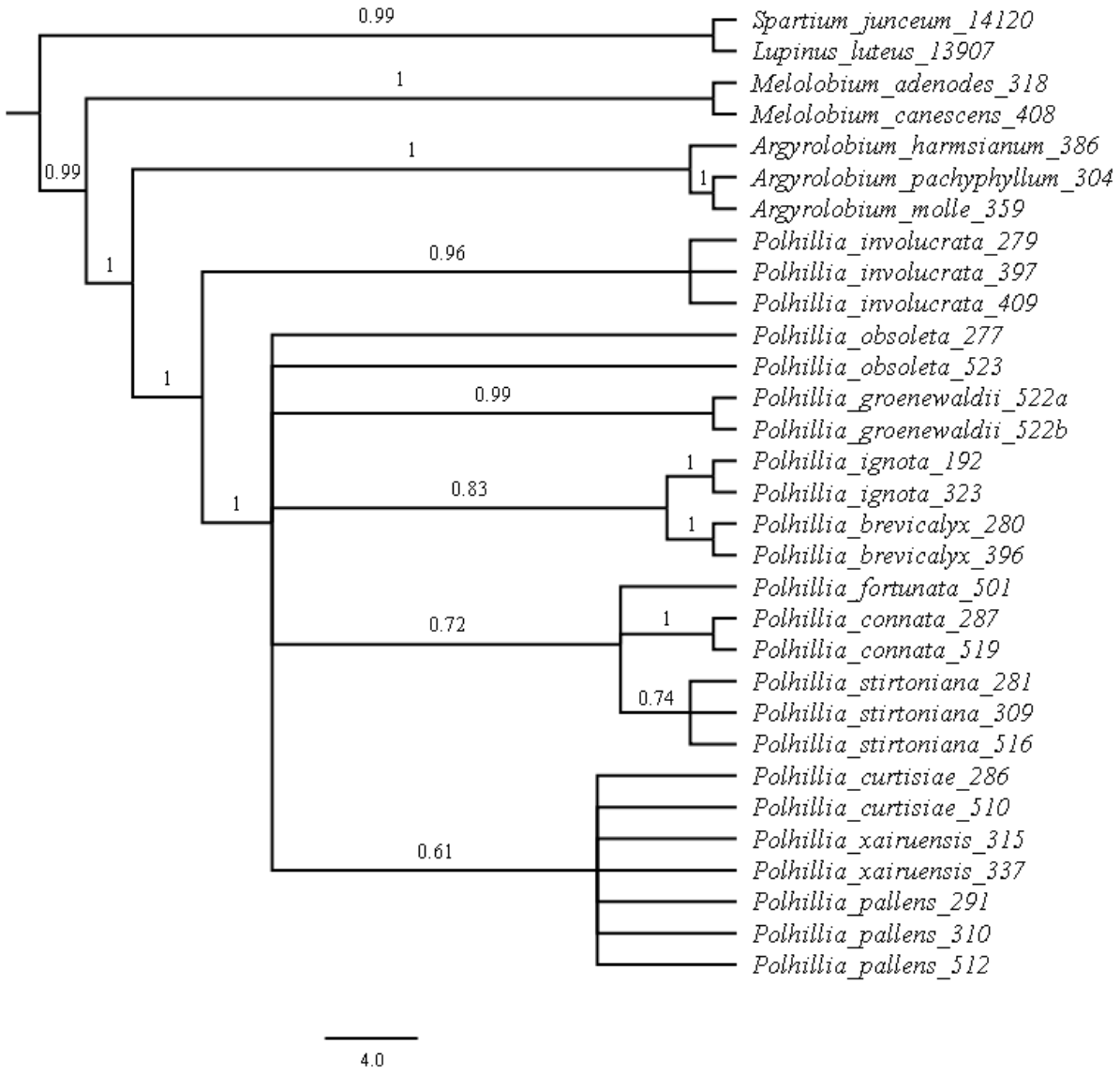

Figure 7: Combined nuclear (ITS) and plastid (trnS-G; psbA-trnH) phylogeny of *Polhillia* and Genisteae outgroups. Values above the branches indicate posterior probabilities.

4.3. Phytogeography

4.3.1. The Greater Cape Floristic Region

In the south-western corner of South Africa lies the Greater Cape Floristic Region (GCFR), a region internationally recognised for its immense botanical diversity within an area of less than 200 000 km^2 (Manning and Goldblatt, 2012; Snijman, 2013). Over 11 400 species occur in this region of which about 70% are endemic. The high species diversity and local endemism seen throughout the region can be attributed, but not limited to, a wide diversity of soil substrates, topographic and climatic variation (Britton *et al*., 2014; Linder, 2003; Manning and Goldblatt, 2012; Snijman, 2013; van der Niet and Johnson, 2009). The GCFR experiences primarily winter rainfall, although annual precipitation varies greatly across the region, with the most rainfall occurring in the SW Cape, and gradually decreasing heading north and inland (Manning and Goldblatt, 2012; Snijman, 2013). The GCFR is home to several different biomes but is best known for the plant diversity within the Fynbos and Succulent Karoo biomes (Manning and Goldblatt, 2012; Snijman, 2013). The Fynbos biome can be further subdivided into four broad vegetation types. They include Fynbos defined by a combination of Proteaceae, Ericaceae and Restionaceae, Renosterveld that typically lacks these elements and is dominated by Asteraceae shrubs and various geophytes, Strandveld and Thicket (Allsopp *et al*., 2014; Rebelo *et al*., 2006). Renosterveld is typically a lowland vegetation type occurring on nutrient rich shale-derived soils, although it also occur at high altitude on some inland escarpments such as the Roggeveld (Clark *et al*., 2011; Snijman, 2013; van der Merwe, 2010; van der Merwe and van Rooyen, 2011). It got its name from the often dominant ‘Renosterbos’ or *Dicerothamnus rhinocerotis* (Cowling *et al*., 1986; Levyns, 1956; Moll *et al*., 1984; Rebelo *et al*., 2006), Renosterveld has, however, been largely transformed for crop agriculture throughout the regions and natural habitat is limited to relatively small fragments (Cowling *et al*., 1986; Kemper *et al*., 1999, 2000; Raimondo *et al*., 2009). These fragments are still home to a wide diversity of plant species though, many of which are highly threatened with extinction due to habitat loss (Curtis *et al*., 2013; Raimondo *et al*., 2009).

4.3.2. Distributions

Polhillia is a small genus consisting of 11 species endemic to the GCFR. *Polhillia* has a centre of diversity in the Overberg region, where 6 of the species occur, with the remaining 5 species occurring in other parts the GCFR (Figure 8). The majority of species are highly localized and occur within a single degree square grid (Table 3). The 3420 degree grid cell is

the most diverse and includes 6 of the 11 *Polhillia* species, 5 of which are endemic to this degree grid. The next most diverse degree square grid cell is 3319, followed by 3321. Only 3 of the 11 *Polhillia* species occur in more than a single degree square grid cell, a further indication of how localized these species are. *Polhillia ignota* and *P. involucrata* are the most widespread species, occurring in four degree square grid cells, although their distributions within these grid cells are limited to isolated populations.

Table 3: Table summarising the distribution of all *Polhillia* species based on the single degree grid reference. Note that eight of the 11 species each only occur in a single grid cell. Also note that grid cell 3420 (Bredasdorp) contains 6 species of the 11 species.

Species	**Degree grid reference**										
	3119	**3120**	**3218**	**3220**	**3317**	**3318**	**3319**	**3320**	**3321**	**3420**	**3421**
P. brevicalyx										X	
P. connata									X	X	X
P. groenewaldii								X			
P. curtisiae										X	
P. fortunata									X		
P. ignota			X		X	X	X				
P. involucrata	X	X		X			X				
P. pallens										X	
P. obsoleta							X				
P. stirtoniana										X	
P. xairuensis										X	

Polhillia species appear to have specific habitat preferences (discussed in more detail in Section 6 and du Preez *et al*., 2019 – Chapter 4). One factor that appears to be consistent is that all species prefer to grow in areas with deep soils without shallow layers of solid rock. *Polhillia* plants never occur on rocky ridges or where plates of shale are exposed on the soil surface. This preference for deeper soils has probably left them vulnerable to land transformation for agriculture, as deep soils are also ideal for ploughing. In the field it appeared as though remnant *Polhillia* populations remained extant due to either inaccessibility for agricultural infrastructure (steep valleys and small hills), or vegetation being preserved for livestock grazing. Very few *Polhillia* populations occur in formally protected areas; therefore, every effort should be made to conserve other populations through stewardship or conservation easement agreements.

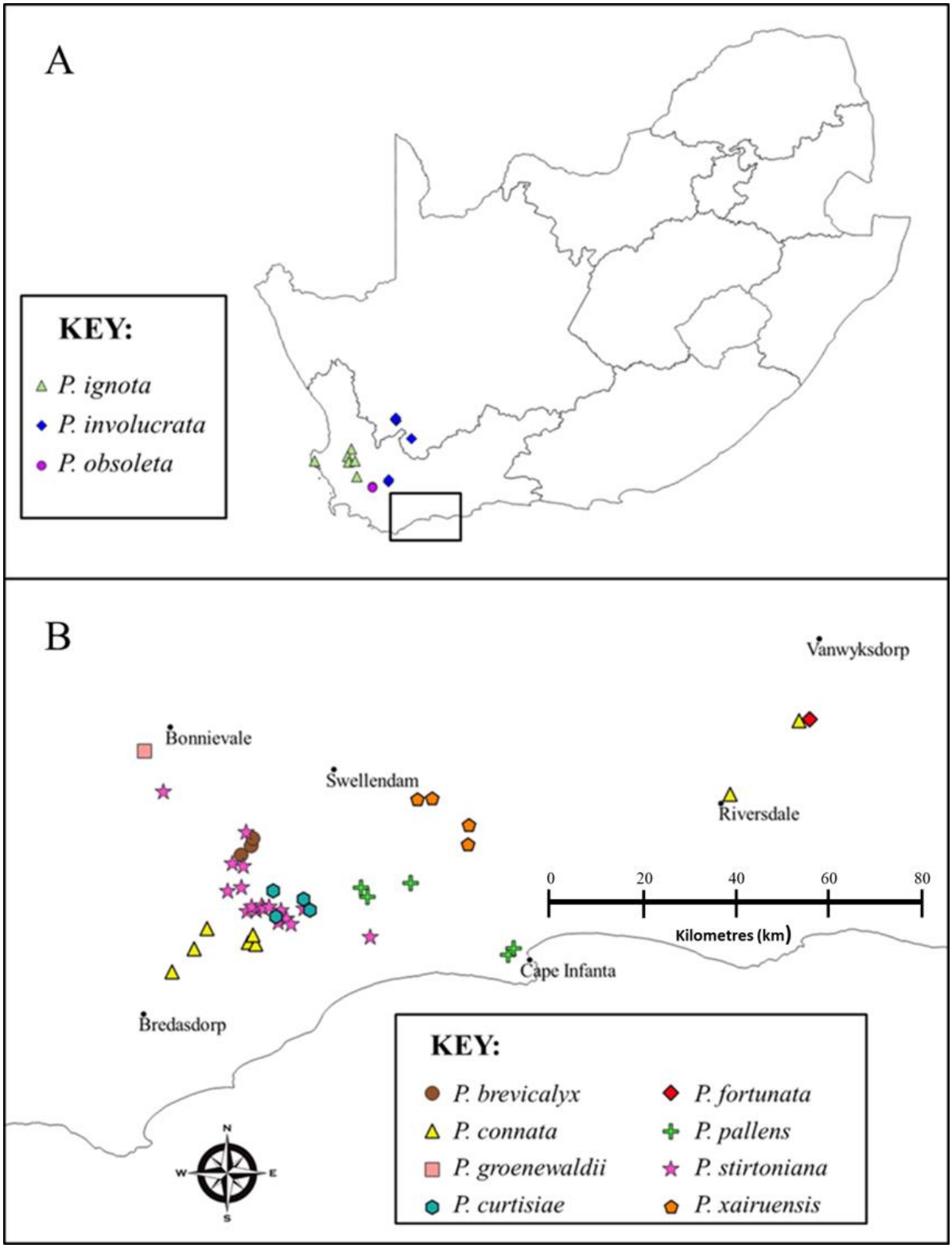

Figure 8: Map showing distribution of all *Polhillia* species recognized in this revision, with particular emphasis on the diversity within and bordering the Overberg region of the Western Cape, South Africa. Note expansion of square in A to map in B.

5. DISCUSSION

Morphological analysis of herbarium specimens and field collected material has shown *Polhillia pallens s.l.* to be a species complex consisting of three morphologically unique species endemic to the Overberg region. We propose that the following two species are recognized as valid species based on morphological evidence: *Polhillia stirtoniana* B.du Preez *sp. nov*. (Section 6, number 10) and *Polhillia xairuensis* B.du Preez *sp. nov.* (Section 6, number 8). *Polhillia stirtoniana* is widespread between Swellendam and Bredasdorp and differs from *P. pallens* in having very narrow falcate leaves, flowers that are typically borne single at branch tips and pods that are weakly plicate. *Polhillia xairuensis* represents the taxon that Stirton (*pers. comm.*) proposed as being morphologically similar to, yet distinct from *P. pallens*. Closer scrutiny has actually showed it to be morphologically more similar to *P. curtisiae* based on having leaves that are densely shaggy hairy and styles that are angled between 45–60°. A further two new species, *Polhillia groenewaldii* B.du Preez *sp. nov.* (Section 6, number 5) and *Polhillia fortunata* B.du Preez *sp. nov.* (Section 6, number 3) were discovered in the Bonnievale and Vanwyksdorp regions respectively during the extensive fieldwork of this study. The former appears to be most similar to *P. obsoleta*, but differs by having styles usually angled at 45–60°, often kinked forward toward the apex, and the height of the curvature is at 1.1–2.4 mm (*versus* style usually angled at about 80° and height of curvature at 2.6–2.8 mm in *P. obsoleta*). *Polhillia fortunata* appears to be a morphological intermediate between *P. ignota* and *Polhillia stirtoniana*. It is characterized by having 2-5 flowers clustered on a pseudo-peduncle, with a densely pubescent, plicate fruit that is also usually falcate. The leaves are more similar to *P. stirtoniana* in that they are densely grey silky hairy rather than green and are smaller and conduplicate relative to the larger and flattened leaves of *P. ignota*. No substantial differences could be found between *P. connata* and *P. canescens* to warrant their continued recognition as two separate species. Floral morphology between these two species is very similar, with the calyx appearing to be identical. They differ substantially in leaf size on herbarium sheets, particularly when comparing type specimens, but field observations showed this trait to be extremely variable; there is naturally a large variation in leaf size based on both seasonal and general plant age variations. We thus believe that *P. canescens* is a synonym of *P. connata s.l.*, although the populations from the Riversdale area needs to be studied in more detail (Section 6, number 6). The circumscription of the remaining species remains the same as sufficient morphological differences were found to maintain their species level circumscriptions.

Based on the cumulative morphological and molecular data, we recognize the following 11 species in the revised genus *Polhillia*:

- *Polhillia brevicalyx* (C.H.Stirt.) B.-E.van Wyk & A.L.Schutte
- *Polhillia connata* (Harv.) C.H.Stirt.
- *Polhillia curtisiae* C.H.Stirt. & Muasya
- *Polhillia fortunata* B.du Preez *sp. nov.*
- *Polhillia groenewaldii* B.du Preez *sp. nov.*
- *Polhillia ignota* Boatwr.
- *Polhillia involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte
- *Polhillia obsoleta* (Harv.) B.-E.van Wyk
- *Polhillia pallens* C.H.Stirt.
- *Polhillia stirtoniana* B.du Preez *sp. nov.*
- *Polhillia xairuensis* B.du Preez *sp. nov.*

Although we attempted to use both morphological and molecular traits in this delimitation, morphology proved more helpful than the rather unresolved molecular phylogenetic reconstructions. Surprisingly all *Polhillia* species sprout after fire, which was unexpected as different fire survival strategies are taxonomically important in several other GCFR legume genera including *Aspalathus*, *Amphithalea*, *Cyclopia*, *Indigofera* L, *Liparia*, *Otholobium*, *Podalyria* Willd., *Psoralea* and *Xiphotheca* Eckl. & Zeyh. (Manning and Goldblatt, 2012; Schutte *et al*., 1995). It is also an important taxonomic character in several other major GCFR families including Proteaceae (Rebelo, 1996; Rourke, 1972), Restionaceae (Linder and Vlok, 1991), Ericaceae and Bruniaceae (Manning and Goldblatt, 2012). Habit, however, proved very useful in species demarcation in *Polhillia*. Stem branching patterns, especially whether there is terminal burst branching or not, was useful in separating otherwise morphologically very similar species in the Overberg. Habit may be influenced by environmental factors and should thus be used with caution in taxonomic treatments. In *Polhillia*, however, although some variation was observed, habit remained constant between species and was useful as a good species-level taxonomic character.

Bark has often been used as a good character for the identification of leguminous trees, for example *Vachellia* Wight & Arn. and *Senegalia* Raf. (Smit, 2012), but has limited proven taxonomic importance is woody leguminous shrubs. *Aspalathus* represents a notable exception. In *Polhillia* bark morphology did not vary between most species, especially the Overberg species. This trait further appeared to vary with the age of plants, rendering it a questionable trait to distinguish between *Polhillia* species.

Leaf characters such as type of pubescence mostly proved to be one of the most informative characters for species level taxonomy of *Polhillia*. This appears to be a general pattern in GCFR legumes, as leaf morphology appears to be more variable than floral morphology in many genera. *Aspalathus* is a great example, as leaves in this genus vary from large, flat unifoliate leaves to minute trifoliolate needle-like leaves (Dahlgren, 1988). Other GCFR genera with very variable leaf morphologies include *Amphithalea* (Granby, 1985; Schutte, 1998), *Rafnia* (Campbell and van Wyk, 2001), *Podalyria* (Schutte-Vlok and van Wyk, 2011), *Cyclopia* (Kies, 1951), *Otholobium* (Stirton, 1989) and *Psoralea* (Bello *et al*., 2017; Dludlu *et al*., 2015). *Polhillia* species can be separated into two groups based on leaf morphology; species with green leaves and species with grey leaves. These colour differences can largely be ascribed to the density of leaf pubescence, but still proved to be a constant and reliable character. Leaf colour was especially useful to distinguish between *P. ignota* and *P. obsoleta*. Overberg *Polhillia* species were reliably separated from the rest based on whether leaf pubescence was shaggy hairy or adpressed sericeous. Seasonal variation in leaf morphology did prove to be a major complication observed in the field. *Polhillia* species appear to respond to environmental factors such as temperature and rainfall in terms of their leaf morphology. Leaves are typically tightly conduplicate during the hot, dry summer months, while opening somewhat during the cooler, wet months. This observed seasonal fluctuation in the appearance of leaves rendered the morphological study of *Polhillia* species based on herbarium voucher specimens only very tricky. We therefore focused on extensive fieldwork and the study of fresh material as much as possible. In this way we could account for seasonal variation and could recognize traits that proved very difficult see in herbarium specimens. Stirton (1986a), in his description of the genus *Polhillia*, included new species such as *P. pallens*. Unfortunately, he mistakenly used photographs of a different species, here described as *P. stirtoniana*, as reference photographs of *P. pallens*. This error probably crept in because the type collection of *P. pallens* was made in February, one of the hottest and driest months that meant that the leaves were tightly closed and appeared to be narrow and similar to *P.*

stirtoniana. The confusion concerning the identities of *P. connata* and *P. canescens* probably also resulted due to natural leaf size variation, and the quality of previously available specimens of these species. Through visiting multiple populations of *P. canescens* during different seasons, we observed substantial variation in leaf size based both on the relative age of individual plants and seasonality. Large, open leaves and small, closed leaves were observed in the type locality of *P. canescens* both in the field and from herbarium voucher specimens. This confirms that there is a substantial natural variation in leaf size and that the leaf morphology of *P. canescens* and *P. connata* is very similar, which led to the synonomy proposed here. Leaf morphology was therefore mostly useful when studying fresh material collected in the cool, winter months and overall proved to be of great taxonomic importance.

Polhillia can be split into two species groups based on the structure of their inflorescences. A pseudo-pedunculate group and a subsessile group could be easily distinguished. All species that lack a pseudo-peduncle (except *P. involucrata*) occur within the southern Overberg region, while pseudo-pedunculate species only occur outside of the southern Overberg. The presence of a pseudo-peduncle is rare among GCFR legumes. It occurs in these few *Polhillia* species, and also in a handful of *Argyrolobium* and *Aspalathus* species (Boatwright, 2010; Boatwright and Cupido, 2011; Dahlgren, 1975; Edwards, 1994; van Wyk and Schutte, 1989). Within the pseudo-pedunculate group, the length of the pseudo-peduncle differed between the 4 species. They were up to 7–8 mm long in *P. obsoleta*, *P. fortunata* and *P. groenewaldii*, but was much longer (up to 15 mm long) in *P. ignota*. The number of flowers per pseudo-peduncle was also important, with *P. obsoleta* and *P. groenewaldii* always having only 1 or 2 flowers, while *P. ignota* had 2–4 flowers and *P. fortunata* had 2-5 flowers per pseudo-peduncle. Pedicel length remained similar in most *Polhillia* species, except in *P. involucrata* and *P. fortunata*, in which it was much longer (6.0 mm and 7.0 mm long, respectively compared to up to 3.5 mm long in all other species). Duminil and Di Michele (2009) flagged pedicel length as a potentially useful taxonomic character among Cape legumes, but this has rarely proven to be the case.

The calyx differences between *Polhillia* species were generally small and not taxonomically very useful, with the exception of *P. connata* and *P. canescens*. Their calyces were identical but differed from all other species in having ventral teeth that were narrowly-triangular to ensiform and converging (versus ventral calyx teeth triangular and rarely converging). This supported the decision to reduce *P. canescens* into synonymy as outlined above. The lack of calyx morphological differences among *Polhillia* species except *P. connata*, is in contrast to

several other Cape legume genera such as *Aspalathus* (Dahlgren, 1988), *Rafnia* (Campbell and van Wyk, 2001), *Podalyria* (Schutte-Vlok and van Wyk, 2011), *Cyclopia* (Kies, 1951) and *Psoralea* (Bello *et al*., 2017; Dludlu *et al*., 2015), where calyx morphology is viewed as an important character in the differentiation between species. In *Aspalathus*, in particular, calyx morphology is often more informative than corolla morphology in discriminating between morphologically similar species (Dahlgren, 1988).

Selection of floral traits include shifts in flower colour, flower size, corolla shape, petal specialization, tube lengths to accommodate various pollinators, aromatic attractants, presentation of rewards for pollinators and methods of presenting sexual organs (Carson, 1985; Castellanos *et al*., 2004; Fenster, 1991; Johnson, 1996; Johnson *et al*., 1998; Pyke, 2016; van der Niet and Johnson, 2012). *Polhillia* species all have a typical zygomorphic papilionoid flower consisting of a standard petal, two wing petals and two keel petals (Lewis *et al*., 2005; Stirton, 1986a; Tucker, 2003; van Wyk and Schutte, 1989). Petal morphology across *Polhillia* species showed limited inter-specific variation. Substantial intraspecific variation led to overlapping traits, rendering the use of corolla morphology of minimal taxonomic importance. Although pollination syndromes were not investigated in this study, pollinators observed visiting *Polhillia* species were restricted to honey-bees and small carpenter bees. The limited interspecific corolla morphological variation observed in closely related *Polhillia* species is consistent with other GCFR legume genera, including *Podalyria* (Schutte-Vlok and van Wyk, 2011), *Indigofera* L. (Schrire *et al*., 2003), Cyclopia (Kies, 1951), *Rafnia* (Campbell and van Wyk, 2001), *Argyrolobium* (Edwards, 1994), *Melolobium* (Moteetee and van Wyk, 2006), *Psoralea* (Bello *et al*., 2017; Dludlu *et al*., 2015) and *Otholobium* (Stirton, 1989), all of which also display limited corolla variation. *Aspalathus* is one of the few genera with diverse floral morphologies, perhaps due to the size of the genus and adaptation to different sized pollinators (Dahlgren, 1988).

The androecium of all *Polhillia* species consisted of a monodelphous staminal tube of 10 stamens. Five short, dorsifixed anthers alternated with 4 basifixed long anthers and one carinal anther, which was typically intermediate in size between long and short anthers. The length of the androecium was dependent on the length of the flowers, which showed both intra- and inter-specific variation. Anther lengths were similar between most species, but *P. connata* had long and short anthers up to 2.5 mm and 1.3 mm in length, respectively. Androecium morphology has not been widely studied in GCFR legumes and represents a knowledge gap that should be investigated. It was found to be of limited importance in

Melolobium with anther sizes varying slightly, with the 5+4+1 anther arrangement remaining constant (Moteetee and van Wyk, 2006). All Genisteae are characterized by having a fused staminal tube and either a 5+5 or 5+4+1 arrangement of anthers (Moteetee and van Wyk, 2006). *Polhillia* anthers, therefore, fit the general patterns seen in SA Genisteae (Edwards, 1994; Moteetee and van Wyk, 2006; Schutte and van Wyk, 1988). We found the arrangement of anthers around the style to be of taxonomic importance in the Overberg species. Anthers were loosely or tightly arranged around the style, and when loosely arranged this was either neat or in a randomly spreading manner. Anthers also shrink substantially when desiccated, so we only used fresh or FAA fixed anthers for measurements. These differences were only evident in fresh flowers, stressing the importance of studying fresh material in addition to herbarium specimens.

The gynoeciums of all *Polhillia* species were largely similar, with only a few taxonomically informative traits emerging. Ovary length varied in relation to the length of the flowers and most species had overlapping dimensions. The number of ovules showed some inter- and intra-specific variation, but putatively closely related species could rarely be separated based on number of ovules. Moteetee and van Wyk (2006) noted that in *Melolobium* ovaries, number of ovules was the only notable gynoecium difference between species. *Aspalathus* species exhibit a wide range of ovary morphologies and ovule numbers, but closely related species are often similar and difficult to separate (Dahlgren, 1988). Style curvature and height of the stigma were used extensively in this study due to the consistency of this character among species, even aiding in the discrimination of closely related species in the Overberg region. Moteetee and van Wyk (2006) also mentioned the importance of style curvature in *Melolobium*, and Dahlgren (1988) found this to be true for *Aspalathus* as well.

Pod morphology in *Polhillia* varied substantially, but closely related species mostly shared very similar pods. As discussed in du Preez *et al*. (2019 – Chapter 2), only *P. involucrata* has dehiscent pods, while all other species have indehiscent fruit. The indehiscent fruited *Polhillia* species can be divided into two groups; the plicate group and the non-plicate group. Species level differences in the plicate group relied on characters such as whether pods were strongly or weakly plicate, pubescence and whether pods were straight or falcate. Differences in the non-plicate pod group were less conspicuous and all pods were linear-oblong in shape and laterally compressed between seeds. Differences were limited to minor differences in length and in some cases differences in pubescence. This was somewhat unexpected, as fruit morphology is regarded as a useful taxonomic trait in legumes. In *Aspalathus*, for example, it

is often used to discriminate between closely related species (Dahlgren, 1988). Fruit morphology is also of taxonomic importance in genera such as *Calobota* (Eckl. & Zeyh.) Boatwr. & B.-E.van Wyk, *Lebeckia* Thunb. and *Wiborgia* Thunb. where floral and leaf morphologies are often similar, but fruit traits vary informatively (Boatwright *et al*., 2009; Dahlgren, 1975).

Polhillia species almost all had morphologically very similar seeds, with inter- and intra-specific variation leaving little room to infer species-level diagnostic characters. Seed size was largely similar between species, except *P. xairuensis* which had seeds of up to 3 mm long compared to the average seed size of 1.6–2.2 mm for all other species. Seed colour varied substantially within and between species, while mottling was restricted to *P. involucrata* and sometimes *P. connata* and *P. pallens*. A similar pattern was found in *Melolobium*, where seed colour varied and no colour was unique to a single species, although more variation in size and mottling patterns were found (Moteetee and van Wyk, 2006). Seed morphology is generally poorly studied in shrubby GCFR legumes and should be investigated in future. Seed morphology in legume tree species has been better studied and was found to be taxonomically informative (Gunn, 1981; Kirkbride *et al*., 2003; Rodrigues *et al*., 2014; Smit, 2012).

Palynologically *Polhillia* species are very homogenous, with only small tectum sculptural details proving unique in *P. connata* and *P. xairuensis*. The tectum was micro-reticulate in all other species, but was coarse-reticulate in *P. connata* and channelled in *P. xairuensis*. Pollen shape was similar across all species, and dimensions were variable. Pollen morphology has been understudied in GCFR legumes. This is surprizing, as studies on non-Cape legumes proved palynology to be informative (González-Vázquez *et al*., 2017; Gunes and Aytug, 2010; Pardo *et al*., 2000; Umdale *et al*., 2017; Zhao *et al*., 2016).

Copious amounts of sclerenchyma in the leaves complicated the anatomical studies of *Polhillia* species. Leaf sections for most species were thus thicker than desired (>20 µm) and leaf sections were often torn during sectioning. Despite these problems, we observed substantial interspecific leaf anatomical variation, rendering leaf anatomy useful for species level taxonomy. Four different mesophyll arrangements were noted among the 11 *Polhillia* species. Isobilateral mesophyll consisting of only palisade mesophyll was found only in *P. ignota*, while isobilateral mesophyll consisting of only spongy mesophyll was found only in *P. xairuensis*. Isobilateral mesophyll with spongy mesophyll in the centre was found in *P.*

obsoleta, *P. connata*, *P. fortunata* and *P. involucrata*. Bifacial mesophyll was found in *P. brevicalyx*, *P. groenewaldii*, *P. curtisiae*, *P. pallens* and *P. stirtoniana*. Although bifacial and isobilateral mesophyll was the dominant form in the Overberg and isobilateral mesophyll dominant outside the Overberg, mesophyll arrangement types were not restricted to either region. The shape of epidermal cells varied from rectangular to conical and papillose, and could be used to distinguish between some species. Only *P. brevicalyx* and *P. involucrata* had rectangular-oval epidermal cells creating a smooth epidermal surface, while all other species had conical or papillose cells on at least one epidermal surface. Overall, leaf anatomy was generally similar between closely related species such as *P. pallens* and *P. curtisiae,* but was useful to discriminate between *P. groenewaldii* and *P. obsoleta* based on different mesophyll arrangements and epidermal morphology.

Previous studies have shown leaf anatomy to be of substantial systematic significance among angiosperms (Jooste *et al*., 2016; Lauterbach *et al*., 2016; Matias *et al*., 2007; Moteetee *et al*., 2002). Leaf anatomy has proven particularly useful, and offered systematically significant traits such as mesophyll structure and shape in *Oxalis* (Jooste *et al*., 2016). Little is known about GCFR legume leaf anatomy, other than intergeneric comparisons published by Boatwright *et al*. (2009). It should definitely be investigated in more detail in future systematic revisions, as it may be used for species-level taxonomy as well as providing possible insights into environmental or habitat shifts and adaptions in other Cape legume genera (Lauterbach *et al*., 2016).

Molecular phylogenetic analysis within the genus *Polhillia* showed poor interspecific resolution with low branch support values and several polytomies. Most of the species we recognize in this revision based on morphology were also resolved in the combined ITS and plastid molecular phylogenetic reconstruction, but with poor branch support values. The unresolved clade containing *P. curtisiae*, *P. pallens* and *P. xairuensis* was not surprising, as these species are morphologically similar, but different enough to recognize as separate species. We though do not trust some species relationships suggested by the molecular phylogeny. The clade containing *P. ignota* and *P. brevicalyx* has 83% posterior probability, but morphologically they are distinct as *P. ignota* is similar to *P. obsoleta* and *P. groenewaldii* in morphology. *Polhillia fortunata*, which is also a member of the plicate pod and pseudo-pedunculate group of *Polhillia*, resolving as sister to *P. connata* is also questionable as this species is clearly related to other Overberg *Polhillia* species. Wide scale molecular data of SA Genisteae such as *Argyrolobium*, *Dichilus* and *Melolobium* are absent

and thus we cannot compare interspecific resolution patterns between these different genera. Poor resolution is, however, rather typical of GCFR legumes. Moiloa *et al*. (2018) reported poor resolution in a study on *Wiborgia*, even after nuclear and plastid data were combined. Widespread poor interspecific phylogenetic resolution was also found among various GCFR genera belonging to the tribe Crotalarieae (Boatwright *et al*., 2008, 2009).

Overall, morphological differences between *Polhillia* species are subtle and inconspicuous, a problem exacerbated by intraspecific variation. Further to this, resolution remained low in all molecular phylogenetic analyses, irrespective of the marker(s) used. Despite these challenges, the collective results of this multidisciplinary assessment enabled the recognition of 11 *Polhillia* species in the GCFR following a morphological species concept. A full taxonomic review for the genus as a whole, and all currently included species, is provided below.

6. TAXONOMIC TREATMENT

At the onset of this study, *Polhillia* included eight species. These species were poorly demarcated, as they were mostly described from very few herbarium specimens and without consideration of both intraspecific and seasonal variation, which affect morphology. *Polhillia* specimens also lose many important characters once preserved as a herbarium specimen, rendering them hard to identify. This has resulted in considerable taxonomic confusion, especially as more specimens started to accumulate from mostly the southern Overberg region in recent years. This small, agricultural region of South Africa is the centre of *Polhillia* diversity. The current study aimed to resolve this taxonomic confusion through exhaustive fieldwork across different seasons, the study of both fresh material and herbarium specimens, and the implementation of a multidisciplinary approach. This enabled us to present an updated taxonomy of *Polhillia*. We now recognize 11 species in the genus, including four new species, and the reduction to synonymy of one formally recognised species.

***6.1. Polhillia* C.H.Stirt.** S Afr. J. Bot. 52: 2 (1986).

Type: *Polhillia waltersii* (C.H.Stirt.) C.H.Stirt. (=*Lebeckia waltersii* C.H.Stirt.)

South Africa, Western Cape, **3320 (Worcester):** Worcester commonage, 30 Jul 1975, Rourke 1484 (K, holotype!; AAU, MO, NBG, S, STE, isotype!).

Plants erect or rarely decumbent, woody shrubs, 0.4–2.5 m tall, sprouting from woody rootstock. *Branches* pale brown to green-yellow, glabrescent; lenticels mostly prominent on old stems; terminal burst branching displayed in some species. *Stipules* deltoid, bifurcate, fused at least around base or up to more than half their length, sheathing stem, sericeous except in *P. involucrata*, 1.0–10.0 mm long. *Leaves* digitately trifoliolate, alternate, pubescent (except in *P. involucrata*), subsessile or rarely shortly petiolate; leaflets conduplicate or partially flattened, linear to narrowly oblanceolate to oblong-obovate, apex acute-apiculate, base cuneate; margins entire; terminal leaflets 4.0–15.0 (–21.0) × 0.7–5.0 (–7.0) mm; lateral leaflets symmetrical, 3.5–15.0 × 0.7–5.0 (–7.0) mm. *Inflorescence* 1–2 (–5) flowers, terminal, subsessile or pseudo-pedunculate, up to 8 mm long; pedicel mostly 2.0–7.0 mm long; bract single at base of pedicel, linear-ensiform, sericeous, caducous, 1.0–1.5 mm long; ebracteolate. *Flowers* bright yellow (pink or white in *P. involucrata*), 7.5–13.0 mm long. *Calyx* campanulate, 3.5–7.5 mm long, bilabiate, lime-green, often flushed maroon-dark pink, shortly sericeous-villous; tube 2.5–5.0 mm long; dorsal lip bifid, teeth triangular, 1.0–3.5 mm long, fused for less than half their length, apex apiculate-acute; ventral lip trifid, teeth triangular or ensiform, 1.0–3.5 mm long, fused for less than half or up to almost their entire length, apex acute-apiculate or rounded; inner tips of teeth pubescent. *Standard petal* 8.0–14.0 mm long; claw linear, 2.0–4.5 mm long; lamina broadly ovate (ovate or broadly elliptical in *P. involucrata*), 6.0–11.0 × 5.5–14.0 mm, apex emarginate, base cordate (broadly attenuate in *P. involucrata*); adaxial standard pubescence variably spread. *Wing petals* 7.0–12.0 mm long, equal to or slightly longer than keel; claw 2.5–4.5 mm long; lamina shape variable, mostly oblong-oblique, 5.0–10.0 × 2.8–5.5 mm, apex rounded-truncate to acute, base auriculate-truncate, depressions present with lamellate sculpturing dorsal above midline, basal-mid, glabrous or pilose along midline. *Keel petals* 6.5–11.8 mm long; claw 2.5–4.5 mm long; lamina mostly lunate, 5.0–10.0 × 2.8–4.5 mm, apex acute-rounded (beaked in *P. connata*), base auriculate to truncate, pilose mostly along front edge, density variable, pocket present, basal along midline. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long and one carinal, basifixed, and 5 short, dorsifixed anthers; filaments fused

forming staminal tube, slit dorsal, unfused portion spreading to erect, loosely or tightly arranged around style. *Pistil* 6.4–10.7 mm long, subsessile; ovary narrowly oblong, 3.6–8.2 mm long, 0.8–1.2 mm wide, sericeous, ovules 7–12; style terete, erect, usually between 45–90° angle, height of curvature 1.1–5.8 mm, glabrous except basally; stigma regular, capitate. *Pods* indehiscent (dehiscent in *P. involucrata*), linear-oblong, plicate or flat, laterally compressed between seeds, straight to slightly falcate, tapering toward the apex and base, sericeous. *Seed* ovate-reniform or squared; testa olive-green to toffy-brown, sometimes almost black, mottled pale orange in *P. involucrata* and occasionally in *P. connata*, smooth; hilum round, recessed, sub-terminal; rim aril inconspicuous; microphyll hidden. *Pollen* tricolpate, oblong to ovate in equatorial view, rounded-triangular in polar view; tectum micro-reticulate to coarsely-reticulate, lumina heterobrochaete, rounded to oblong, pollen kit ample, intra-luminary bacules present.

6.2. Diagnostic traits

A combination of several strong characters diagnose the genus *Polhillia* within in the tribe Genisteae in South Africa. These include the absence of true peduncles, with flowers single, in terminal clusters or pseudo-pedunculate. Other SA Genisteae genera typically have flowers borne on true peduncles, except for a handful of *Argyrolobium* species. The two stipules are always fused, at least around the base, and sheath the stem. Other SA Genisteae typically have two unfused stipules that do not sheathe the stem. Most *Polhillia* species have subsessile leaves, while other SA Genisteae genera typically have distinctly petiolate leaves. *Polhillia* species also lack bracteoles, which are common in other SA Genisteae genera. All *Polhillia* species, except *P. involucrata*, have indehiscent fruit, which is very unique, as all other SA Genisteae have dehiscent fruit.

6.3. Key to *Polhillia* species

1a Leaves glabrous, flowers pink or white, fruit dehiscent _____
_____ subgen. *Roseopolhillia* 11. *Polhillia involucrata*

1b Leaves pubescent, flowers yellow, fruit indehiscent _____ subgen. *Polhillia*__ 2

2a Flowers less than 9 mm long, leaves distinctly petiolate _____ 1. *Polhillia brevicalyx*

2b Flowers more than 9 mm long, leaves subsessile _____ 3

3a Leaves usually green, fruit usually strongly plicate, pseudo-peduncles present _____4

4a Flowers 2–4 (up to 5) on pseudo-peduncle, pods densely pubescent _____ 5

5a Flowers 2–4, pseudo-peduncle up to 15 mm long, leaves bright green _____ 2. *Polhillia ignota*

5b Flowers 2–5, pseudo-peduncle up to 7 mm long, leaves silky grey sericeous _____ 3. *Polhillia fortunata*

4b Flowers usually solitary or sometimes paired on pseudo-peduncle, pods glabrescent _____ 6

6a Pods strongly plicate, ovules 12, distribution around Worcester _____ 4. *Polhillia obsoleta*

6b Pods weakly plicate to linear, ovules usually 10, distribution near Bonnievale _____ 5. *Polhillia groenewaldii*

3b Leaves silvery, fruit laterally compressed between seeds, pseudo-peduncles absent _____ 7

7a Calyx longer than 6 mm, keel rostrate, flowers in clusters of up to 4 _____ 6. *Polhillia connata*

7b Calyx shorter than 6 mm, keel lunate, flowers single or in clusters of 2 _____ 8

8a Leaves shaggy hairy, style angled at 45–60°_____ 9

9a Shrubs 0.5–1 m tall, pods velutinous, distribution around Spitskop, W of Breede River ____ 7. *Polhillia curtisiae*

9b Shrubs 1–1.8 m tall, pods sericeous, distribution around Suurbraak, E of Breede River ___ 8. *Polhillia xairuensis*

8b Leaves adpressed sericeous-canescent, style angled at 70–90° _____ 10

10a Leaves more than 1 mm wide, oblong-obovate and straight, shrub 1.5–2.5 m tall, sparsely branching terminally _____ 9. *Polhillia pallens*

10b Leaves less than 1 mm wide, linear-lanceolate and arcuate, shrub 0.8–1.2 m tall, with terminal burst branching _____ 10. *Polhillia stirtoniana*

6.4. Taxonomy

1. Polhillia brevicalyx (C.H.Stirt.) B.-E.van Wyk & A.L.Schutte, Kew Bulletin 44: 3 (1989); *Argyrolobium brevicalyx* C.H.Stirt. S, Afr. J. Bot. 50: 4 (1984). Type: South Africa, Western Cape, **3420 (Bredasdorp):** Flats along watercourse 1 km SSE of Uitvlug farm, along road between Bredasdorp and Swellendam, 15 km SW of Swellendam (–AB), 1 Oct 1983, *Burgers 3188* (K, holotype!; STE, isotype!).

Plant a low growing, rounded, procumbent or decumbent shrub to 0.5 m tall, often forming wide circular bushes, sprouting from woody rootstock. *Branches* pale brown, sericeous on young branches, becoming glabrescent on older branches; lenticels prominent. *Stipules* 1.0–3.0 mm long, deltoid, bifurcate, fused only near base, densely sericeous. *Leaves* digitately trifoliolate, conduplicate, bright green, petiole shorter than lamina, 1.0–3.0 mm long, leaflets narrowly obovate, slightly falcate, margin entire, glabrous to sparsely hairy adaxially, sericeous abaxially and along margins, apex apiculate, base cuneate, terminal and lateral leaflets equal, 2.5–8.0 ×1.0–3.0 mm. *Inflorescence* terminal cluster of 1–3 (–5) flowers, peduncle absent, pedicel up to 3.0 mm long; bract ± 1.5 mm long, linear-subulate, sericeous, caducous; ebracteolate. *Flowers* bright yellow, small, 7.5–8.5 mm long. *Calyx* bilabiate, 3.5–4.5 mm long, shortly sericeous, tube 2.5–3.0 mm long, dorsal lip bifid, ± 1.5 mm long, fused to about half their length, teeth triangular, ventral lip trifid, teeth narrowly-triangular, ± 1.5 mm long, fused for less than half their length, inner tips of teeth sericeous. *Standard petal* 8.0–8.5 mm long; claw linear, 2.0–2.5 mm long; lamina ovate, 6.0–6.5 × 5.5–7.0 mm, apex emarginate, base cordate; back of standard pilose only along midline. *Wing petals* 7.0–7.8 mm long, equal to or slightly longer than keel; claw ± 2.5 mm long; lamina oblong, 5.0–6.0 × 2.8–3.0 mm, apex obtuse-rounded, base truncate-auriculate, elliptical depression and lamellate sculpturing dorsi-basal above midline; glabrous. *Keel petals* 6.5–7.5 mm long; claw ± 2.5 mm long; lamina obliquely-lunate, 5.0–6.0 × 3.0–3.5 mm, apex obtuse-rounded, base truncate, pilose along front edge; pocket present, basal along midline. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long, basifixed anthers, 0.9–1.0 mm long, and one carinal anther, 0.8 mm long, and five short, dorsifixed anthers, 0.5–0.6 mm long; filaments fused forming staminal tube, 5.6–6.2 mm long, slit dorsal, unfused portion remaining equidistant from style, sub-erect, neatly arranged around style. *Pistil* 6.4–7.5 mm long, subsessile; ovary narrowly oblong, 3.6–4.4 × 0.8–0.9 mm, sericeous, ovules 7; style terete, erect, usually at about 80–90° angle, height of curvature 1.7–2.3 mm, glabrous except

basally; stigma regular, capitate. *Pod* indehiscent, oblong, flattened, laterally compressed between seeds, green-brown, sericeous, 11.0–20.0 × 3.0–3.5 mm. *Seed* ovate-reniform; testa olive-green to brown, smooth, 1.7–2.1 × 1.6–2.0 mm, 1.4–1.7 mm thick; hilum round, recessed, sub-terminal; rim aril inconspicuous; microphyll hidden. *Pollen* tricolpate, oblong in equatorial view, rounded-triangular in polar view, polar apices rounded-truncate, size 29.0–34.0 × 16.0–20.0 µm, semi-tectate, tectum micro-reticulate to finely-reticulate, lumina heterobrochaete, rounded to oblong, pollen kit ample, intra-luminary bacules present.

Flowering time: September to October

Figure 9: Composite plate of *Polhillia brevicalyx*. 1 – Flower front view; 2 – Flower side view; 3 – Flower bottom view; 4 – Wing petals; 5 – Keel petals; 6 – Androecium; 7 – Gynoecium; 8 – Pods; 9 – Close-up view of leaves and stipules; 10 – Habit. Scale bars: 2 mm. Photographs by B. du Preez.

Etymology

The specific epithet “brevicalyx” refers to the notably small calyx of this species in comparison with other *Polhillia* species.

Diagnostic characters

Polhillia brevicalyx (Figure 9) is distinguished from other *Polhillia* species by its decumbent or procumbent, spreading shrub (*versus* erect shrubs in other species), its comparatively small flowers (< 9 mm long *versus* 10 mm or longer in other species), having a distinct petiole (*versus* leaves subsessile in other species), and it has 7 ovules (*versus* 9–12 ovules in all other species, except *P. involucrata*).

Distribution, ecology and conservation status

This species is known only from a small area around the type locality (Figure 10). Plants grow mostly just above the riverbanks of the Sout River on the farm Uitvlug, east of the R319 between Swellendam and Bredasdorp. This species grows in small isolated patches extending along a stretch of roughly 6 km. A thorough population survey located about 180 plants, which is double the number previously recorded (Raimondo *et al*., 2009), but it remains critically endangered. This species grows in deep clay in Eastern Rûens Shale Renosterveld (FRs 13) bordering on saline Cape Lowland Alluvial Vegetation (AZa 2) (Mucina and Rutherford, 2006). The Red Data List status of this species is assessed as CR B1ab(iii,v)+B2(iii,iv); C2(ii) following the latest IUCN categories and criteria (IUCN, 2012).

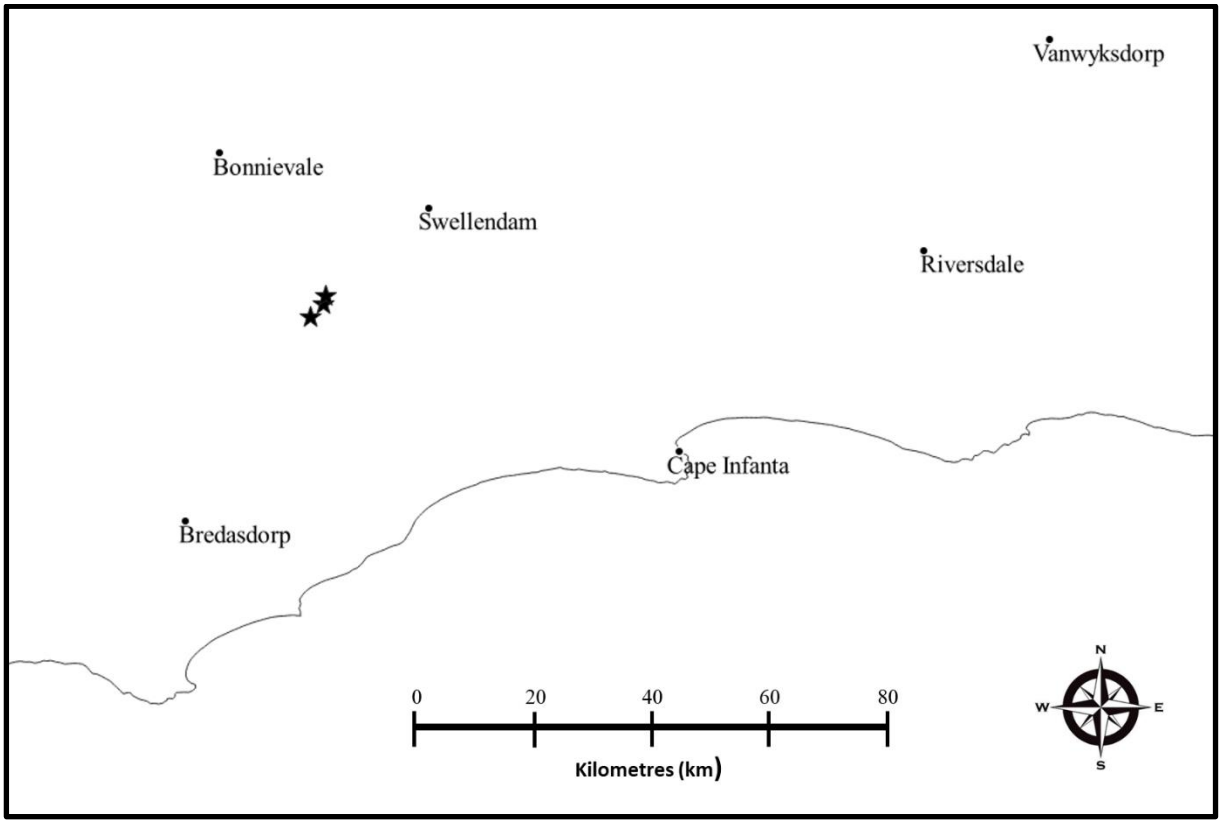

Figure 10: Distribution of *Polhillia brevicalyx*.

Additional specimens examined

South Africa. WESTERN CAPE: **3420 (Bredasdorp):** Uitvlug Farm, Lower Breede River Valley (–AA), 13 Oct 2003, *CREW 31* (NBG); Uitvlugt farm, just off R319 (–AB), 19 May 2017, *du Preez 280* (BOL); 8 Oct 2017, *du Preez 353* (BOL); 17 Oct 2017, *du Preez 395* (BOL); Uitvlugt farm, North of Bredasdorp (–AB), 9 Oct 1986, *B.-E. van Wyk 2100* (JRAU), *2101* (JRAU), *2102* (JRAU), *2103* (JRAU); Farm: Uitvlugt, between Bredasdorp and Swellendam, 1km west of homestead (–AB), 18 Dec 1986, *S. Streicher s.n. sub A.L. Schutte 389* (JRAU); Witkop farm behind dam, just N of Uitvlugt (–AB), 17 Oct 2017, *du Preez 396* (BOL, NBG); Uitvlugt farm, Bredasdorp area (–AB), 26 Nov 2011, *Stirton & Curtis 13567* (BOL).

2. Polhillia ignota Boatwr., S. Afr. J. Bot. 76 (2010). Type: South Africa, Western Cape, **Vredenburg (3317):** Between Vredenburg and "Hoetjesbaai" [Houdjies Bay] (–BB), 5 Sept 1928, *Hutchinson 253* (K, holotype!).

Plant an erect, rounded shrub 1.0–1.5 m tall, sprouting from woody rootstock. *Branches* shiny yellow-brown, densely sericeous on young branches, becoming glabrescent on older branches; lenticels absent; terminal burst branching present. *Stipules* 1.0–3.0 mm long, deltoid, bifurcate, fused, sericeous. *Leaves* digitately trifoliolate, subsessile, bright green, leaflets elliptic-narrowly obovate, margin entire, involute, adaxial surface sparsely hairy, abaxial surface and margin sericeous, apex obtuse, base cuneate, terminal leaflet 4.0–12.0 × 1.0–2.5 mm, lateral leaflets 4.0–10.0 × 1.0–2.5 mm, mature leaflets flattened, young growth conduplicate. *Inflorescence* terminal clusters of (1–) 2–4 flowers, pseudo-pedunculate or rarely subsessile, pseudo-peduncles up to 15.0 mm long; pedicel up to 3.0 mm long; bract single at base of pedicel, linear, 1.0–1.5 mm long, sericeous, caducous; ebracteolate. *Flowers* bright yellow, 10.0–12.0 mm long. *Calyx* campanulate, 4.0–5.0 mm long, bilabiate, lime-green, villous; tube 3.0–3.5 mm long; dorsal lip bifid, teeth broadly triangular, 1.0–1.5 mm long, fused for less than one third of their length, tips acute; ventral lip trifid, fused, teeth triangular, 1.0–1.5 mm long, fused for about two thirds of their length, converging, tips apiculate; inner tips of teeth pubescent. *Standard petal* 10.0–12.0 mm long; claw linear, 3.5–4.5 mm long; lamina broadly ovate, 7.5–9.0 × 9.0–12.0 mm, apex emarginate, base cordate; back of standard short sericeous on upper parts only. *Wing petals* 8.0–10.0 mm long, equal in length to keel; claw 3.0–4.0 mm long; lamina lunate, 6.0–8.0×3.5–4.5 mm, apex obtuse, base truncate to shortly auriculate, two lunate and one oval depression with lamellate sculpturing,

dorsal above midline, basal-mid; glabrous. *Keel petals* 8.0–10.0 mm long; claw 3.5–4.2 mm long; lamina lunate, 6.0–7.0×3.0–3.5 mm, apex obtuse-rounded, base truncate, almost completely glabrous, finely pilose along apical front edge, pocket present, basal along midline. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long, basifixed anthers, 1.1–1.5 mm long, and one carinal anther, 1.0 mm long, and five short, dorsifixed anthers, 0.7 mm long; filaments fused forming staminal tube, 8.0–8.7 mm long, slit dorsal, unfused portion remaining closely united, sub-erect, tightly arranged around style. *Pistil* 8.1–10.1 mm long, subsessile; ovary narrowly oblong, 5.0–6.7 mm long, 0.8–1.0 mm wide, sericeous, ovules 12; style terete, erect, usually at about 70–90° angle, height of curvature 2.9–3.3 mm, glabrous except basally; stigma regular, capitate. *Pod* indehiscent, oblong, plicate, laterally compressed between seeds, straight to slightly falcate, tapering toward the apex and base, woody, sericeous, pale-brown, 17.0–20.0 × 3.0–4.0 mm. *Seed* ovate-reniform; testa olive-green to dark-brown, smooth, 2.0–2.5 × 1.8–2.3 mm, 1.6–1.9 mm thick; hilum round, recessed, sub-terminal; rim aril inconspicuous; microphyll hidden. *Pollen* tricolpate, oblong in equatorial view, rounded-triangular in polar view, polar apices truncate-rounded, size 27.0–30.0 × 16.0–19.0 µm, semi-tectate, tectum micro-reticulate to coarsely-reticulate, lumina heterobrochaete, rounded to oblong, pollen kit ample, intra-luminary bacules present.

Flowering time: August to October

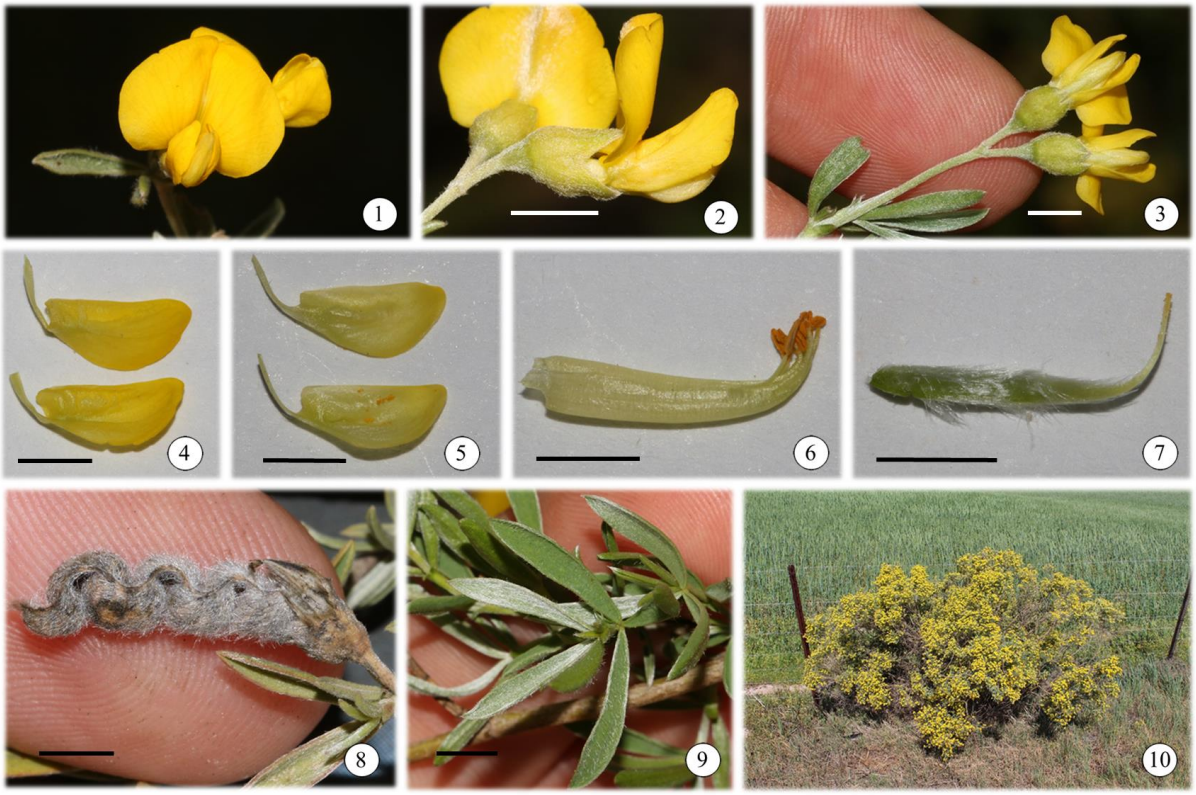

Figure 11: Composite plate of *Polhillia ignota*. 1 – Flower front view; 2 – Flower side view; 3 – Flower bottom view; 4 – Wing petals; 5 – Keel petals; 6 – Androecium; 7 – Gynoecium; 8 – Pod; 9 – Close-up view of leaves and stipules; 10 – Habit. Scale bars: 4 mm. Photographs by B. du Preez.

Etymology

The origin of the specific epithet "ignota" is unknown, but it was probably used as this distinctive species was ignored by taxonomists until it was eventually described Boatwright (2010).

Diagnostic characters

Polhillia ignota (Figure 11) is morphologically most similar to *Polhillia obsoleta* and was only recently described by Boatwright (2010), and is distinguished from *P. obsoleta* by its bright green leaves (*versus* blue-green leaves); pseudo-peduncles that are up to *ca*. 15.0 mm long and carry up to 4 flowers (*versus* pseudo-peduncles of only up to *ca*. 8.0 mm long carrying only 1 or occasionally 2 flowers); pale brown, densely pubescent pods (*versus* russet brown, pubescent-glabrescent pods); ventral calyx tips converging (*versus* ventral calyx tips spreading); wing and keel petals basally truncate (*versus* wing and keel petals basally auriculate); wing petals lunate with 3 dorsal basal-mid depressions (*versus* wing petals

obliquely oblong with 2 dorsal basal-mid depressions). The geographical distribution of this species is also distinct, as it occurs only in the Swartland, whereas *P. obsoleta* only occurs in the Breede River valley around Worcester.

Distribution, ecology and conservation status

The type collection of this species is from the Vredenburg area along the west coast of South Africa. This area has, however, been extensively transformed by agricultural, industrial and urban development and the species has, most likely, now become extinct in this area. Extensive field surveys throughout this region proved unsuccessful. Its current distribution is restricted to four localities in Swartland Shale Renosterveld (FRs 9) (Mucina and Rutherford, 2006), where it grows on shales overlaid by alluvial sediment. Two of the locations are in the northern Swartland region around Eendekuil (16 plants) along with a single plant growing in the fence line along the N7 highway 5 km north of Piketberg (Figure 12). Two further populations were recently discovered south of there in the Voëlvlei Nature Reserve and southwest of Porterville. Additional individuals from these two new populations brought the known global population size to roughly 230 plants. Over 90% of the Swartland Shale Renosterveld has been transformed for agriculture (Ebrahim *et al*., 2016; Mucina and Rutherford, 2006), but we are optimistic more populations may be discovered through more focussed searches in potentially suitable habitat. The Red Data List status of this species was assessed as EN A2c; B1ab(i,ii,iii,iv,v)+B2ab(i,ii,iii,iv,v); C2a(i); D following the latest IUCN categories and criteria (IUCN, 2012).

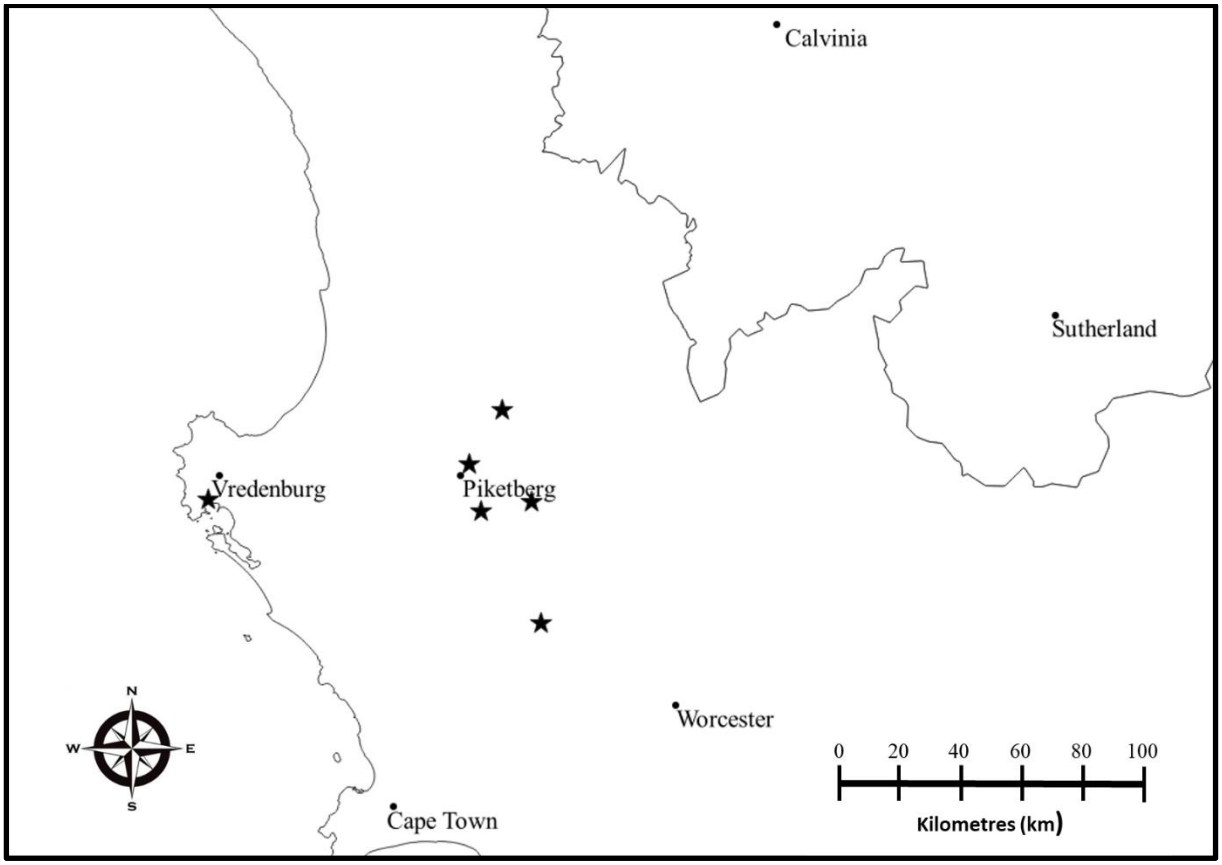

Figure 12: Distribution of *Polhillia ignota*.

Additional specimens examined

South Africa. WESTERN CAPE: **3218 (Clanwilliam):** Farm Goedehoop, just south of Eendekuil (–DB), 27 Apr 2016, *du Preez 186* (BOL); 11 Sept 2016, *du Preez 192* (BOL); 1 Oct 2017, *du Preez 349* (BOL); N7. About 5km north of Piketberg (–DD), 4 Sept 2017, *du Preez 323* (BOL, K, NBG, PRE, MO). **3318 (Cape Town):** Prope Porterville (–BB), 27 Dec 1907, *Edwards s.n. sub BOL13438* (BOL). **3319 (Worcester)**: Voëlvlei NR, in and next to western firebreak (–AC), 8 Sept 2018, *du Preez 526* (BOL).

3. Polhillia fortunata B.du Preez, *sp. nov*.

Type: South Africa, Western Cape, **3321 (Ladismith):** Vanwyksdorp District, Farm Waterval, about 1.5 km east of homestead along road to Herbertsdale (–CD), 23 Sept 2018, *du Preez 564* (BOL, holotype!; K, NBG, MO, PRE, SCHG, STE, isotype!)

Plant an erect shrub up to about 2.0 m tall, sprouting from woody rootstock. *Branches* silky-sericeous when young, older branches unknown. *Stipules* deltoid, bifurcate, fused and sheathing stem, sericeous, 2.0–4.0 (–6.0) mm long. *Leaves* digitately trifoliolate, grey-green, silky-sericeous, subsessile; leaflets conduplicate, oblanceolate, apex apiculate, base cuneate; margins entire; terminal leaflet 3.0–8.0 × 1.5–3.5 mm; lateral leaflets symmetrical, 3.0–7.5 × 1.0–3.5 mm. *Inflorescence* 2–5 flowers, terminal, pseudo-pedunculate, pseudo-peduncles up to 7.0 mm long; pedicel up to 7.0 mm long; bract single at base of pedicel, linear, sericeous, caducous, ± 1 mm long; ebracteolate. *Flowers* yellow, 9–12 mm long. *Calyx* campanulate, 3.5–4.8 mm long, bilabiate, lime-green, shortly villous; tube 2.5–3.5 mm long; dorsal lip bifid, teeth broadly triangular, 1.0–1.5 mm long, fused for less than one third of their length, apex acute; ventral lip trifid, teeth triangular, 1.0–1.5 mm long, fused for ± half their length, apex acute; inner tips of teeth pubescent. *Standard petal* 11.5–13.5 mm long; claw linear, 3.5–5.0 mm long; lamina broadly ovate, 9.0–10 × 11.5–13.5 mm, apex emarginate, base cordate; back of standard shortly sericeous only on and around apical half of midline. *Wing petals* 10.1–11.1 mm long, slightly longer than keel; claw 3.0–4.0 mm long; lamina obovate, 8.4–9.3 × 3.7–4.1 mm, apex obtuse, base auriculate, triangular and oblong depression with lamellate sculpturing dorsal above midline, basal-mid, glabrous. *Keel petals* 9.2–10.5 mm long; claw 3.0–4.0 mm long; lamina broadly lunate, 7.1–7.7 × 3.5–4.2 mm, apex acute-squared, base auriculate-truncate, pilose along front edge, pocket present, basal along midline. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long, basifixed anthers, 1.1–1.4 mm long, and one carinal anther, 0.9–1.0 mm long, and five short, dorsifixed anthers, 0.5–0.6 mm long; filaments fused forming staminal tube, 6.8–9.6 mm long, slit dorsal, unfused portion spreading somewhat, sub-erect, loosely arranged around style. *Pistil* 9.2–10.9 mm long, subsessile; ovary narrowly oblong, 5.9–7.0 mm long, 1.0–1.2 mm high, sericeous, ovules 10–12; style terete, sub-erect to erect, usually at about 70–90° angle, height of curvature 2.5–3.0 mm, glabrous except basally; stigma regular, capitate. *Pod* indehiscent, oblong, plicate, laterally compressed between seeds, straight or falcate toward apex, tapering toward the apex and base, sericeous, olive green before ripe, 21.0–25.0 × 2.5–3.0 mm, ripe pods unknown. *Seed* unknown. *Pollen* unknown.

Flowering time: August to October

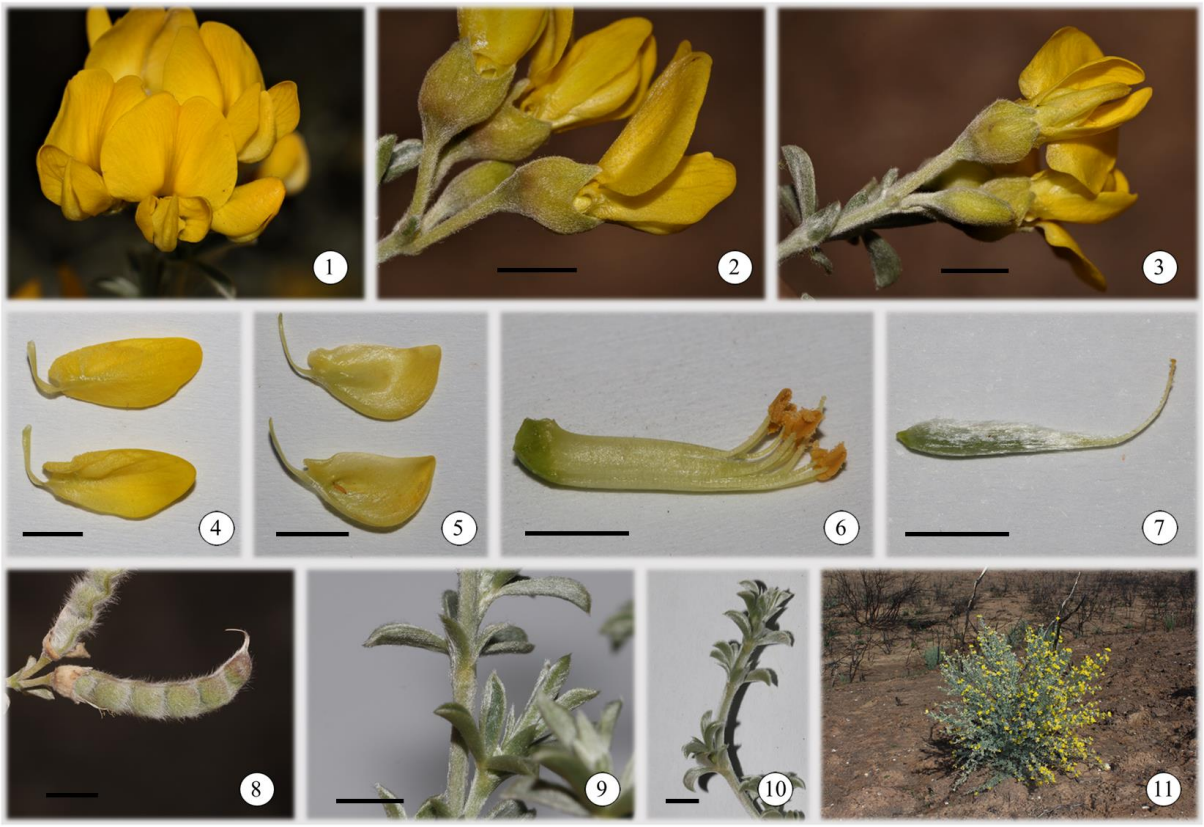

Figure 13: Composite plate of *Polhillia fortunata*. 1 – Flower front view; 2 – Flower side view; 3 – Flower bottom view; 4 – Wing petals; 5 – Keel petals; 6 – Androecium; 7 – Gynoecium; 8 – Pod; 9 – Close-up of leaves and stipules; 10 – Close-up of recurved leaves; 11 – Habit. Scale bars: 4 mm. Photographs by B. du Preez.

Etymology

The specific epithet is a direct translation from Latin meaning “luck”. This epithet was selected as the discovery of this species was very lucky. The area in which this species occurs was burnt in a massive fire for the first time in nearly 100 years in February 2018. I had driven past this exact location in mid-2017 and did not notice these plants. On return home in the winter of 2018 from a search for a previously known *Polhillia* site nearby, I by chance noticed grey bushes sprouting from the ashes, which turned out to be this new species. It was not in flower, and the luck continued when I returned in spring only to find that plants had sprouted profusely and flowered within a year post-fire, despite the severe drought.

Diagnostic characters

Polhillia fortunata (Figure 13) appears to be morphologically similar to *P. obsoleta* and *P. ignota*, but also shares morphological characters seen in Overberg *Polhillia* species. The presence of pseudo-peduncles comprised of 2–5 flowers points to an affinity with *P. obsoleta* and *P. ignota*, but the leaves are densely silvery pubescent, similar to species such as *P. pallens* and *P. xairuensis*. *Polhillia fortunata* bears the strongest resemblance to the Swartland endemic *P. ignota* based on the dense arrangement of flowers on a pseudo-peduncle and the densely pubescent and plicate fruit. It, however, differs from *P. ignota* in having falcate fruit (*versus* straight), leaves up to 8.0 × 3.5 mm, conduplicate, densely silky sericeous and arcuate (*versus* leaves up to 12.0 × 2.5 mm, flattened, green sericeous and straight), and pseudo-peduncle up to 7.0 mm long (*versus* up to 15 mm long). This species is unique among *Polhillia* species in often having recurved leaf tips. It also has comparatively long pedicels of up to 7.0 mm long, while pedicels of other *Polhillia* species never exceed 3.5 mm, with the exception of *P. involucrata* that has a pedicel of up to 6.0 mm long.

Distribution, ecology and conservation status

This species is known only from the type location (Figure 14) where it grows in Montagu Shale Renosterveld (FRs 7) on south facing slopes and along a seasonal riverbed (Mucina and Rutherford, 2006). It appears to be highly localized, as much of the surrounding habitat appears to be unsuitable, even though of the area is still in a natural state. This area has botanically generally been poorly explored and other sub-populations may still be found (Vlok, *pers. comm.*). This population does not appear to be facing any direct threats at present, as most of this area is used for wildlife breeding and there are no exotic tree species growing in the area. The potential impacts of climate change on the long-term survival of this species remain unknown, but it appears to be well adapted to its native arid habitat. Due to the arid nature of the area, fire is infrequent and the last fire in that area before the fire of January/February 2018 was in the early 1920's based on Muir's collection of *P. connata* in 1925. We thus suggest generation lengths of between 80 and 100 years. This single population consists of roughly 300–400 plants that were sprouting after the recent fire, while some seedling recruitment was also observed. Their fate remains unclear under the ongoing drought currently being experienced in the area. The Red Data List status of this species is assessed as VU D1 (IUCN, 2012).

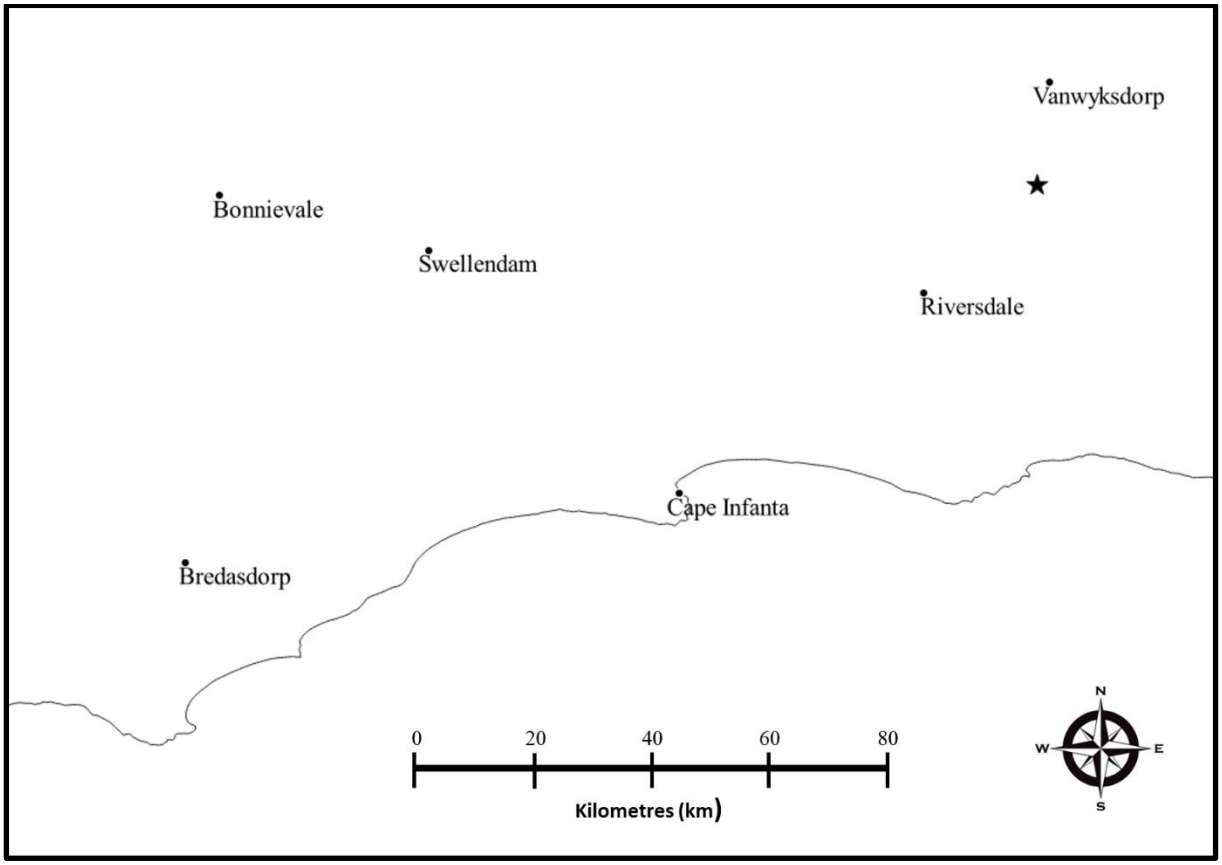

Figure 14: Distribution of *Polhillia fortunata*.

Additional specimens examined

South Africa. WESTERN CAPE: **3321 (Ladismith):** Vanwyksdorp District, Farm Waterval, about 1.5km east of homestead along road to Herbertsdale (–CD), 29 Jun 2018, *du Preez 501* (BOL).

4. Polhillia obsoleta (Harv.) B.-E.van Wyk, Bothalia 22: 1 (1992). *Argyrolobium obsoletum* Harv. Flora Capensis 2: 70 (1862). Type: Cape, without precise locality, *Thunberg s.n. sub UPS-THUNB 16504* (UPS, holotype).

Aspalathus sericea sensu Thunb. Flora capensis 574 (1823) non DC.

Lebeckia waltersii C.H.Stirt. Bothalia 13: 3-4 (1981). *Polhillia waltersii* (C.H.Stirt.) C.H.Stirt., S. Afr J. Bot 52: 4 (1986). Type: South Africa, Western Cape, **3319 (Worcester):** Worcester commonage 1.5 miles north-west of town (–CB), 30 Jul 1975, *Rourke 1484* (K, holotype!, NBG, isotype!).

Plant an erect, rounded shrub up to 1.5 m tall, sprouting from woody rootstock. *Branches* pale green-brown to golden; lenticels prominent on old stems; with terminal burst branching. *Stipules* deltoid, bifurcate, fused and sheathing the stem, sericeous, 1.0–3.5 mm long. *Leaves* digitately trifoliolate, sericeous, subsessile; leaflets conduplicate, partially flattened, narrowly oblanceolate, apex acute, base cuneate; margins entire; terminal leaflet 4.0–11.0 (–16.0) × 1.0–2.5 (–3.5) mm; lateral leaflets symmetrical, 3.5–10.5 (–15.0) × 1.0–2.5 (–3.5) mm. *Inflorescence* 1–2 flowered, terminal, pseudo-pedunculate, pseudo-peduncles up to 8.0 mm long; pedicel 2.0–3.0 mm long; bract single at base of pedicel, linear, sericeous, caducous, ± 1.0 mm long; ebracteolate. *Flowers* yellow, 10.0–11.0 mm long. *Calyx* campanulate, 3.5–4.0 mm long, bilabiate, lime-green, sometimes flushed maroon, shortly sericeous-villous; tube ± 2.5 mm long; dorsal lip bifid, teeth broadly triangular, 1.0–1.5 mm long, fused for less than one third of their length, apex apiculate-acute; ventral lip trifid, teeth triangular, 1.0–1.5 mm long, fused for ± half their length, apex acute; inner tips of teeth pubescent. *Standard petal* 9.5–11.0 mm long; claw linear, ± 3.5 mm long; lamina broadly ovate, 6.5–8.0 × 9.5–11.0 mm, apex emarginate, base cordate; back of standard shortly sericeous only on and around apical half of midline. *Wing petals* 9.0–10.0 mm long, equal to or slightly longer than keel; claw 3.5–4.0 mm long; lamina obliquely oblong, 7.5–8.0 × 3.0–4.0 mm, apex rounded-truncate, base auriculate, triangular and oblong depression with lamellate sculpturing dorsal above midline, basal-mid, glabrous. *Keel petals* 8.5–9.5 mm long; claw 3.5–4.0 mm long; lamina broadly-lunate, 6.5–7.5 × 3.2–3.8 mm, apex obtuse, base rounded-auriculate, pilose along front edge, pocket present, basal along midline. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long, basifixed anthers, 1.3–1.4 mm long, and one carinal anther, 1.1–1.2 mm long, and five short, dorsifixed anthers, 0.7 mm long; filaments fused forming staminal tube, 6.9–8.0 mm long, slit dorsal, unfused portion spreading

somewhat, sub-erect, loosely arranged around style. *Pistil* 8.1–9.0 mm long, subsessile; ovary narrowly oblong, 5.5–6.3 mm long, ± 1.0 mm wide, sericeous, ovules 12; style terete, erect, usually at about 80° angle, height of curvature 2.6–2.8 mm, glabrous except basally; stigma regular, capitate. *Pod* indehiscent, oblong, plicate, laterally compressed between seeds, straight to slightly falcate, tapering toward the apex and base, woody, sericeous between folds, sericeous to glabrescent elsewhere, russet brown-chestnut, 16–23 × 3.0–4.0 mm. *Seed* ovate-reniform; testa olive-green to toffy-brown, sometimes finely brown-dotted, smooth, 1.7–2.5 × 1.6–2.2 mm, 1.4–1.9 mm thick; hilum round, recessed, sub-terminal; rim aril inconspicuous; microphyll hidden. *Pollen* tricolpate, oblong to ovate in equatorial view, rounded-triangular in polar view, polar apices truncate-rounded, roughly 24–33 × 13–20 µm in size, semi-tectate, tectum micro-reticulate to finely-reticulate, lumina heterobrochaete, rounded to oblong, pollen kit ample, intra-luminary bacules present.

Flowering time: (Feb) June to August

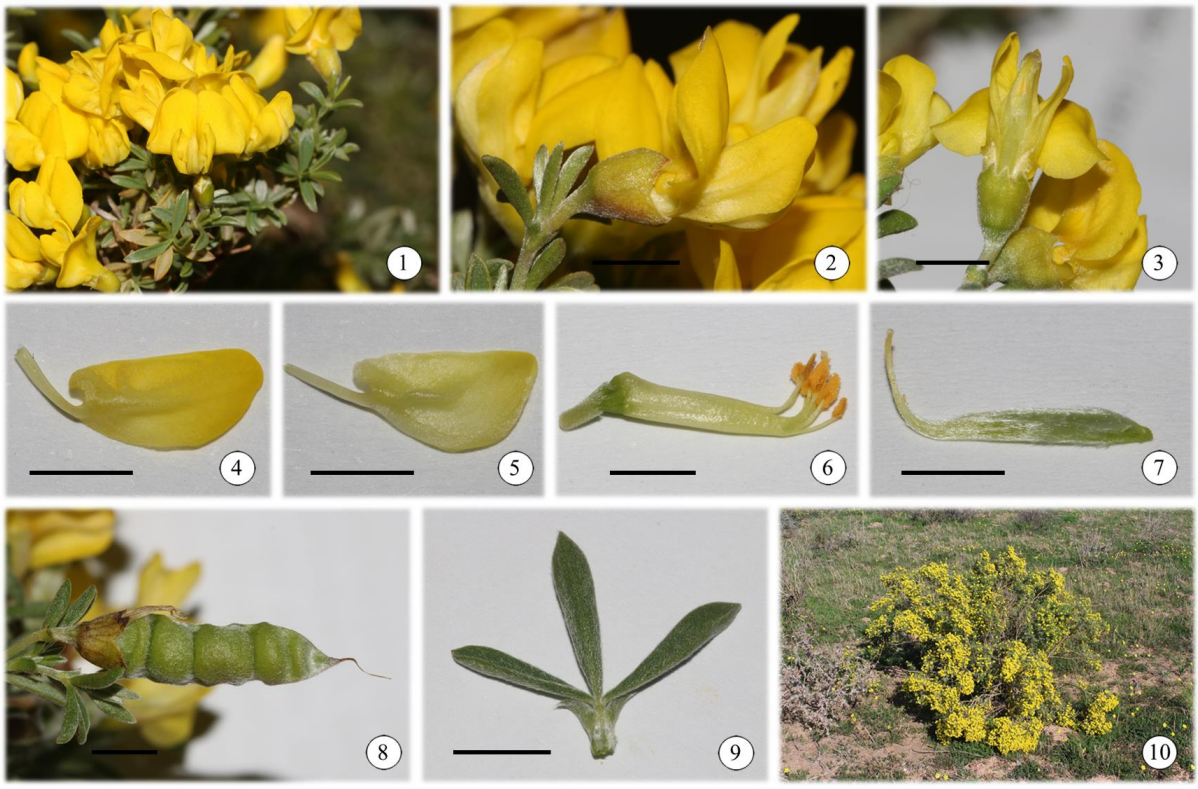

Figure 15: Composite plate of *Polhillia obsoleta*. 1 – Flower front view; 2 – Flower side view; 3 – Flower bottom view; 4 – Wing petal; 5 – Keel petal; 6 – Androecium; 7 – Gynoecium; 8 – Pod; 9 – Close-up of leaf and stipules; 10 – Habit. Scale bars: 4 mm. Photographs by B. du Preez.

Etymology

The origin of the specific epithet "obsoleta" is unknown as Harvey (1862) did not substantiate his reasoning for the epithet when he described this species originally as *Argyrolobium obsoletum* Harv.

Diagnostic characters

Polhillia obsoleta (Figure 15) is morphologically most similar to *P. ignota* but is best distinguished by its blue-green (*versus* leaves bright green) leaves; pseudo-peduncles that are up to 8.0 mm long bearing 1 or 2 flowers (*versus* pseudo-peduncles up to 15 mm long with up to 4 flowers); pods russet brown and pubescent-glabrescent (*versus* pods pale brown and densely pubescent); ventral calyx tips spreading (*versus* ventral calyx tips converging); wing and keel petals basally auriculate (*versus* wing and keel petals basally truncate); wing petals obliquely oblong with 2 dorsal basal-mid depressions (*versus* wing petals lunate with 3 dorsal basal-mid depressions). The geographical distribution of this species is also distinct, as it occurs only in the Breede River valley around Worcester, whereas *P. ignota* only occurs in the Swartland.

Distribution, ecology and conservation status

This species is endemic to a small area around the town of Worcester in the Breede River valley (Figure 16). Much of its former habitat has been lost to the expansion of the town and it remains threatened by the possibility of further development. This species is now restricted to a few fragments of Breede Alluvium Renosterveld (FRa 1) along the urban fringe of Worcester (Mucina and Rutherford, 2006). The largest remaining population is at the Worcester Airfield, which is home to roughly 500 plants. A smaller population of roughly 200 plants occurs just south of the N1 highway. This area is proposed to be formally conserved as a conservation offset for a nearby development, thus offering this species some protection from future development. This will be the only formally protected population of this species. The Red Data List status of this species is assessed as EN A2c; B1ab(iii,v)+B2ab(iii,v) following the latest IUCN categories and criteria (IUCN, 2012).

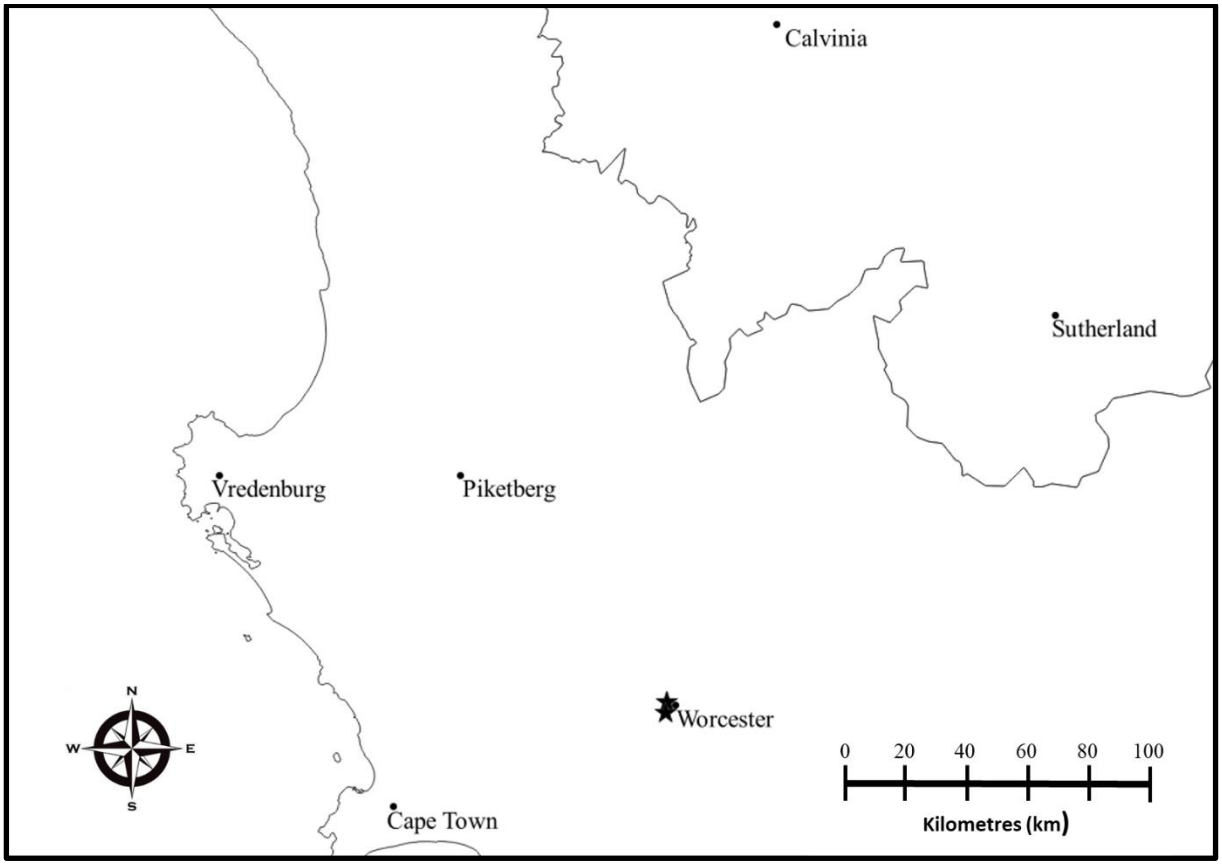

Figure 16: Distribution of *Polhillia obsoleta*.

Additional specimens examined

South Africa. WESTERN CAPE: **3319 (Worcester):** Worcester commonage 1.5 miles north-west of town (–CB), 30 Jul 1975, *Rourke 1484* (NBG); 22 Feb 1981, *C.J. Burgers 2636* (NBG); Veld near Worcester drive-in cinema (–CB), 15 Nov 1980, *I.B. Walters 1919* (NBG); 10 Sept 1976, *I.B. Walters 1507* (NBG); 24 Jul 1975, *I.B. Walters 190* (NBG); 20 Sept 1976, *I.B. Walters s.n. sub NBG113326* (NBG); 17 Jul 1981, *I.B. Walters 1918* (NBG); 5 Feb 1980; *B.-E. van Wyk 204* (NBG); Worcester West (–CB), 10 Jul 1975, *I.B. Walters 58* (NBG); New golf club house site (–CB), 28 Aug 1985, *Bayer & Forrester 4904* (NBG); Worcester turn-off from N1 (–CB), 6 Aug 2003, *Manning 2847* (NBG); Worcester C.P. (–CB), 19 Jul 1962, *I.B. Walters 7* (NBG); 27 Jul 1968, *I.B. Walters 7* (NBG); Worcester airfield, near hangers (–CB), *H.M. Walters 623* (NBG); Worcester airfield, between runways (–CB), 21 Apr 2017, *du Preez 277* (BOL); 30 Jul 2017, *du Preez 306* (BOL, PRE); Worcester, veld between N1 and water reservoir (–CB), 1 Nov 2007, *Cowell, Bennett & Pekeur 3554* (NBG); Worcester (–CB), 12 Oct 2009, *Muasya, Stirton & Curtis 4104* (BOL); 4 Jul 1987, *B.-E. van Wyk 2701*

(JRAU); Near Worcester (–CB), 28 Sept 1978, *Esterhuysen 35048* (BOL); Worcester, S of N1 and west of mall (–CB), 8 Aug 2018, *du Preez 523* (BOL).

5. Polhillia groenewaldii B.du Preez, *sp. nov.*

Type: South Africa, Western Cape, **3320 (Montagu):** Bonnievale Region, Bokdam farm, NE of Stormsvlei Pass (–CC), 8 Aug 2018, *du Preez 522* (BOL, holotype!; K, NBG, MO, PRE, isotype!)

Plant an erect, rounded shrub up to 1.2 m tall, sprouting from woody rootstock. *Branches* grey-brown, rough, densely fissured; lenticels prominent; with terminal burst branching. *Stipules* deltoid, bifurcate, fused and sheathing stem, sericeous, 1.5–3.0 mm long. *Leaves* digitately trifoliolate, blue-green, sericeous, subsessile; leaflets conduplicate, partially flattened, narrowly oblanceolate, apex acute, base cuneate; margins entire; terminal leaflet 4.0–8.0 × 1.0–2.5 mm; lateral leaflets symmetrical, 4.0–7.5 × 1.0–2.5 mm. *Inflorescence* 1–2 flowered, terminal, pseudo-pedunculate, pseudo-peduncles up to 10.0 mm long; pedicel up to 3.0 mm long; bract single at base of pedicel, linear-subulate, green, sericeous, caducous, ± 1.0 mm long; ebracteolate. *Flowers* yellow, 9.5–11.0 mm long. *Calyx* campanulate, 3.7–4.5 mm long, bilabiate, lime-green, shortly villous; tube 2.5–3.5 mm long; dorsal lip bifid, teeth broadly triangular, 1.0–1.5 mm long, fused for less than a third of their length, apex acute; ventral lip trifid, teeth triangular, 0.9–1.3 mm long, fused for ± two thirds of their length, apex acute; inner tips of teeth pubescent. *Standard petal* 9.5–11.5 mm long; claw linear, 2.8–3.8 mm long; lamina broadly ovate, 7.2–8.1 × 8.5–10.3 mm, apex emarginate, base cordate; back of standard shortly sericeous only on and around apical half of midline. *Wing petals* 8.6–11.0 mm long, equal to or slightly longer than keel; claw 3.3–4.5 mm long; lamina narrowly oblong-lunate, 7.0–8.0 × 2.5–3.2 mm, apex acute-obtuse, base auriculate, triangular and lanceolate depression with lamellate sculpturing dorsal above midline, basal-mid, glabrous. *Keel petals* 8.8–10.0 mm long; claw 3.0–4.3 mm long; lamina obliquely-lunate, 6.5–7.5 × 3.2–3.8 mm, apex obtuse, base auriculate, pilose along front edge, pocket present, basal along midline. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long, basifixed anthers, 1.2–1.4 mm long, and one carinal anther, 1.0–1.1 mm long, and five short, dorsifixed anthers, 0.55–0.65 mm long; filaments fused forming staminal tube, 7.0–8.6 mm long, slit dorsal, unfused portion spreading somewhat, sub-erect, loosely arranged around style. *Pistil* 8.9–10.2 mm long, subsessile; ovary narrowly oblong, 6.0–7.0 mm long, 0.8–1.0

mm wide, sericeous, ovules usually 10, sometimes 8 or 11; style terete, sub-erect, usually at about 45–60° (–80°) angle, often kinked forward toward apex, height of curvature 1.1–2.4 mm, glabrous except basally; stigma regular, capitate. *Pod* indehiscent, oblong, partially plicate, laterally compressed between seeds, straight or falcate toward apex, tapering toward the apex and base, sparsely pubescent, dirty-brown drying to cream-brown, 21.0–27.0 × 3.0–3.2 mm. *Seed* ovate-reniform; testa olive-green to dark toffy-brown, smooth, 1.9–2.4 × 1.7–2.2 mm, 1.5–1.9 mm thick; hilum round, recessed, sub-terminal; rim aril inconspicuous; microphyll hidden. *Pollen* not seen.

Flowering time: July to August

Figure 67: Composite plate of *Polhillia groenewaldii*. 1 – Flower front view; 2 – Flower side view; 3 – Flower bottom view; 4 – Wing petals; 5 – Keel petals; 6 – Androecium; 7 – Gynoecium; 8 – Pod; 9 – Close-up of leaves and stipules; 10 – Habit. Scale bars: 4 mm. Photographs by B. du Preez.

Etymology

The specific epithet “groenewaldii” honours Mr Jannie Groenewald who first discovered this species and notified the authors thereof. The naming of this species after Jannie is in honour of the incredible work he has done on increasing the knowledge of plants in the Overberg region through the discovery of new species and also new populations of threatened plant species. This is also in recognition of the tremendous help he provided during fieldwork conducted for this study. His passion and love for plants will serve as a driving force to protect this species and hopefully find more populations thereof.

Diagnostic characters

Polhillia groenewaldii (Figure 17) is morphologically similar to *P. obsoleta*, but also displays morphological traits typical of Overberg *Polhillia* species. The presence of pseudo-peduncles with 1–2 flowers, leaves that are blue-green in colour and partially conduplicate suggest a close affinity to *P. obsoleta*. The most definitive difference is that the fruits are elongate and partially plicate, 21.0–27.0 × 2.8–3.2 mm (*versus* short and strongly plicate, 16.0–23.0 × 3.0–4.0 mm); pods dirty-brown to cream-brown and fibrous (*versus* pods russet-brown and woody). Other notable differences include ovaries with 10, sometimes 8 or 11 ovules (*versus* ovaries with 12 ovules); style usually at about 45–60° (–80°) angles, often kinked forward toward apex, height of curvature 1.1–2.4 mm (*versus* style usually at about a 80° angle, height of curvature 2.6–2.8 mm); wing petal lamina narrowly oblong-lunate, 7.0–8.0 × 2.5–3.2 mm, apex acute-obtuse (*versus* wing petal lamina obliquely oblong, 7.5–8.0 × 3.0–4.0 mm, apex rounded-truncate); bark pale grey-brown, densely fissured and lenticels prominent (*versus* bark pale green-brown to golden, often relatively smooth and lenticels prominent only on older stems).

Distribution, ecology and conservation status

This species grows on south facing slopes in Breede Shale Renosterveld (FRs 8), which is fairly arid and dominated by Karroid shrubs and succulents such as *Tulista pumila* (Lam.) G.D. Rowley (Mucina and Rutherford, 2006). This species is presently known from a single locality on a road verge between Stormsvlei and Bonnievale (Figure 18). Only 4 plants were found at this location and although much of the area is still in a natural state, it is uncertain how much of this may be suitable for this species. This area is generally poorly explored and other sub-populations may still be found. A wide scale search of this area is essential as a population total of 4 plants render the long-term survival of this species near impossible. The

population does not appear to be facing any direct threats at present, but the four plants are growing in an old canal up on a road verge. It is uncertain whether these plants are only persisting here due to the collection of rainwater in this canal and that the remaining area has become too arid for the species to survive. Climate change may thus also potentially place further strain on the future survival of this species. The Red Data List status of this species is assessed as CR B1ab(i,ii,iii,iv,v)+B2ab(i,ii,iii,iv,v); C1+C2a(i,ii); D following the latest IUCN categories and criteria (IUCN, 2012).

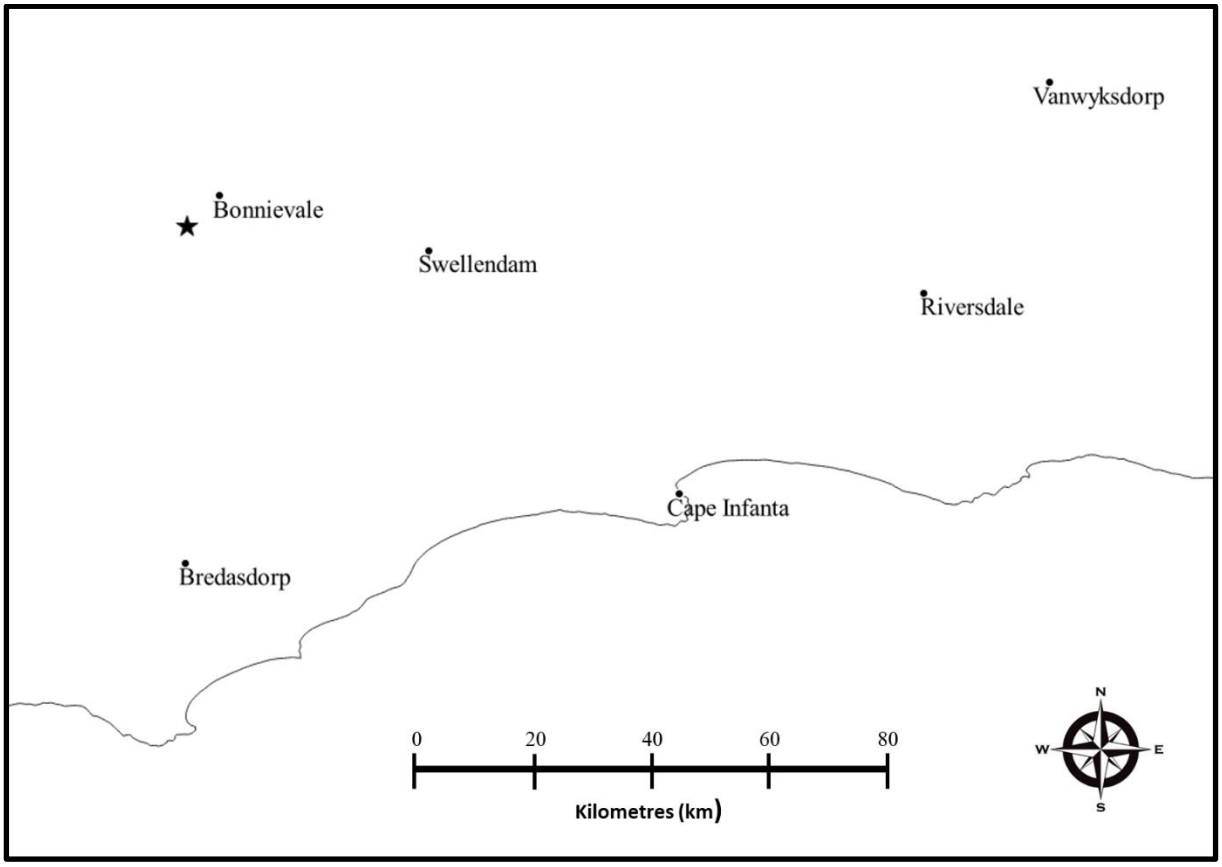

Figure 18: Distribution of *Polhillia groenewaldii*.

6. Polhillia connata (Harv.) C.H.Stirt, S. Afr. J. Bot. 52: 2 (1986). Type: Cap. Bon. Spei, without precise locality, *Thom 37* (K, holotype!).

Argyrolobium connatum Harv., Fl. Cap. 2: 72 (1862).

Genista connata (Harv.) Briq., Étude Cytises Alpes Mar. 119 (1894).

Polhillia canescens C.H.Stirt. S Afr. J. Bot. 52: 2 (1986), *syn. nov*. Type: South Africa, Western Cape, **3420 (Bredasdorp):** Adamskop, north of Bredasdorp, 23 Sept 1982, *Bayer 3104* (NBG, holotype!; K, isotype!).

Plant an erect, lax, multi-stemmed shrub up to 1.8 m tall, sprouting from woody rootstock. *Branches* greyish-brown, young stems sericeous-villous, old stems glabrescent, lenticels prominent. *Stipules* 5.0–10.0 mm long, deltoid, bifurcate, fused, sheathing stem, imbricate, canescent-sericeous. *Leaves* digitately trifoliolate, canescent-sericeous, grey, subsessile; leaflets oblanceolate, conduplicate, flattened during wet season; apex acute-apiculate, base cuneate, margin entire; terminal leaflet 7.0–21.0 × 2.0–5.0 (–7.0) mm, lateral leaflets symmetrical, 6.0–21.0 × 2.0–5.0 (–7.0) mm. *Inflorescence* terminal axillary clusters of 2–4 flowers, restricted to upper branches, subsessile; pedicel to 4.0 mm long; bract single at base of pedicel, 1.0–1.5 mm long, linear, sericeous, caducous; ebracteolate. *Flowers* yellow, 11.5–13.0 mm long. *Calyx* campanulate; 6.0–7.5 mm long; bilabiate, yellow-green, sericeous; tube 3.0–4.0 mm long; dorsal lip bifid, teeth triangular, 3.0–3.5 mm long, fused for less than one third of lobe length, apex acute; ventral lip trifid, teeth narrowly triangular-ensiform, 3.0–3.5 mm long, fused to ± half of lobe length, tips converging on one another, apex acute-apiculate; inner tips of teeth pubescent. *Standard petal* 11.5–14.0 mm long; claw linear, 3.0–4.5 mm long; lamina broadly ovate, 8.5–11.0 × 10.0–14.0 mm, apex emarginate, base cordate, adaxial surface shortly and sparsely pilose, back of standard sericeous all over except along basal flanks. *Wing petals* 10.5–12.0 mm long, equal to or slightly longer than keel; claw 3.5–4.0 mm long; lamina oblong-lunate, 8.5–10.0 × 4.0–5.5 mm, apex acute-obtuse, base minutely auriculate; oval and lunulate depressions with lamellate sculpturing dorsi-basal above midline, pilose along midline to completely glabrous. *Keel petals* 10.0–11.8 mm long; claw 3.5–5.0 mm long; lamina lunate-oblanceolate, beaked, sub-rostrate, 7.5–8.0 × 3.5–4.5 mm, apex obtuse, base truncate-auriculate; pilose along ventral half, dense along front ventral edge; pocket present, dorsal along midline. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long, basifixed anthers, 2.4–2.5 mm long, and one carinal anther, 2.2–2.4 mm long, and five short, dorsifixed anthers, 1.2–1.3 mm long; filaments fused

forming staminal tube, 8.4–9.2 mm long, slit dorsal, unfused portion remaining closely united, sub-rostrate, tightly arranged around style. *Pistil* 9.3–10.7 mm long, subsessile; ovary narrowly oblong, 5.4–6.0 mm long, 1.2–1.4 mm high, sericeous, ovules 9; style terete, erect-rostrate, usually at 80–90° angle, height of curvature 4.8–5.8 mm, sericeous, becoming glabrous towards apex; stigma regular, capitate. *Pod* indehiscent, oblong, laterally compressed between seeds, straight to slightly falcate, tapering towards apex and base, sericeous, pale-brown, 30.0–45.0 × 4.0–5.0 mm. *Seed* ovate-reniform; testa olive-green to dark-brown, rarely marbled pale-orange, smooth; 2.3–2.7 × 1.9–2.4 mm, 1.7–1.9 mm thick; hilum round, recessed, sub-terminal; rim aril inconspicuous; microphyll hidden. *Pollen* tricolpate; oblong to ovate in equatorial view, rounded-triangular in polar view, polar apices rounded; roughly 22.0–33.0 × 19.0–21.0 µm in size; semi-tectate, tectum micro-reticulate to coarsely-reticulate, lumina heterobrochaete, rounded to oblong; pollen kit ample; intra-luminary bacules present.

Flowering time: September to October

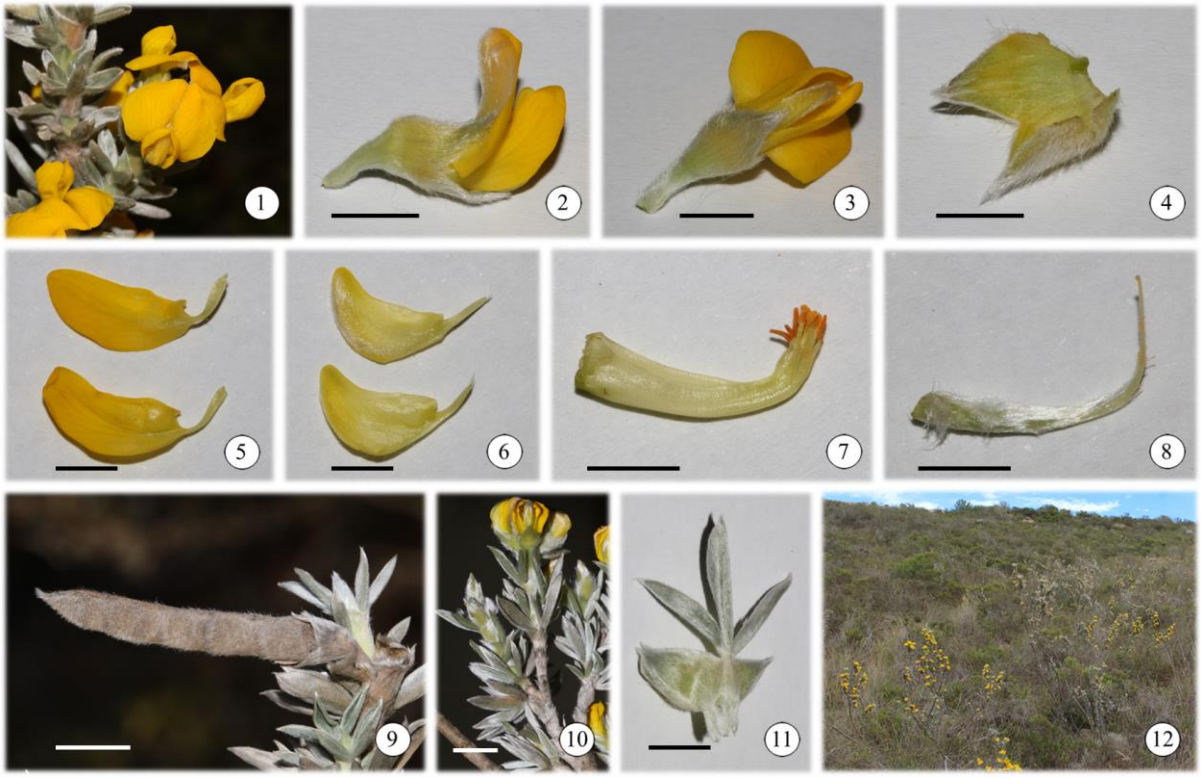

Figure 19: Composite plate of *Polhillia connata*. 1 – Flower front view; 2 – Flower side view; 3 – Flower bottom view; 4 – Calyx; 5 – Wing petals; 6 – Keel petals; 7 – Androecium; 8 – Gynoecium; 9 – Pod; 10 – Close-up of leaves and stipules; 11 – Close-up of a single leaf and stipules; 12 – Habit. Scale bars: 4 mm (except images 9 and 10 – Scale bars: 10 mm). Photographs by B. du Preez.

Etymology

The specific epithet “connata” refers to the stipules that are connate and sheathe the stems in this species, although this is also a character of all other *Polhillia* species.

Diagnostic characters

Polhillia connata (Figure 19) is easily distinguished from other *Polhillia* species by its erect, lax growth form (*versus* rounded erect shrubs in other species); rostrate-beaked keel (*versus* rounded, lunate in other species); large, flattened canescent-grey leaves up to 7.0 mm wide (*versus* leaves usually less than 3.5 mm wide).

Having studied the type specimens of *Polhillia connata* and *Polhillia canescens*, as well as material collected during the extensive fieldwork for this study, it became apparent how confusion has been caused in the taxonomy of this species. Leaf size is very variable in this species, influenced by both the age of the plants and seasonal conditions. The type material of *P. connata* is almost 200 years old and appears to have been collected from a depauperate plant with fairly small leaves, which were closed, while the type of *P. canescens* was collected from a very healthy individual with large, open leaves. Flower morphological features were difficult to assess due to the age of the type specimen and the lack of available flowers, but comparable traits were within reasonable levels of variation to belong to the same species. The calyces of the two taxa were identical, with the unique ventral trifid calyx teeth narrowly triangular-ensiform, 3.0–3.5 mm long, fused to ± half of lobe length, and the tips converging on one another. Although very little detail is known about the type specimen collected by Rev. Thom in the 1820’s, it is plausible that he would have travelled in the wider southern Overberg area and probably came across this species in the Bredasdorp area. Stirton (1986a) speculated that the collection may have come from near Caledon, where Rev. Thom was based, but no *Polhillia* has been recorded from this area. Based on this anecdotal evidence, in combination with the morphological similarities and the observed natural environmental leaf size variation, we here reduce *Polhillia canescens* into synonymy under *P. connata*.

Distribution, ecology and conservation status

This species is endemic to the Overberg region, where it is known from 6 localities north of Bredasdorp (Figure 20). It grows on shale-derived soils in Central and Eastern Rûens Shale

Renosterveld (FRs 12 & FRs 13) on rocky slopes (Mucina and Rutherford, 2006). Plants were found on most aspects, but appeared to favour northerly facing slopes and gullies. A thorough assessment of the population located roughly 1500 plants. The *P. connata* populations in the Riversdale area remain a mystery, both in terms of its taxonomy and whether these populations still exist. Only 3 collections were made in this area, the most recent being in 1925. All collections are without precise locality details. John Muir collected this species on both sides of the Langeberg Mountains during the 1920's. Despite extensive searches in the course of this study, none of these populations could be relocated. The majority of the lowland Renosterveld in the area has been ploughed for agriculture and it is likely that these populations have become extinct since the last collection in 1925. The Red Data List status of this species is assessed as VU A2c; B1ab(iii,iv,v)+B2ab(iii,iv,v); C2a(i) following the latest IUCN categories and criteria (IUCN, 2012).

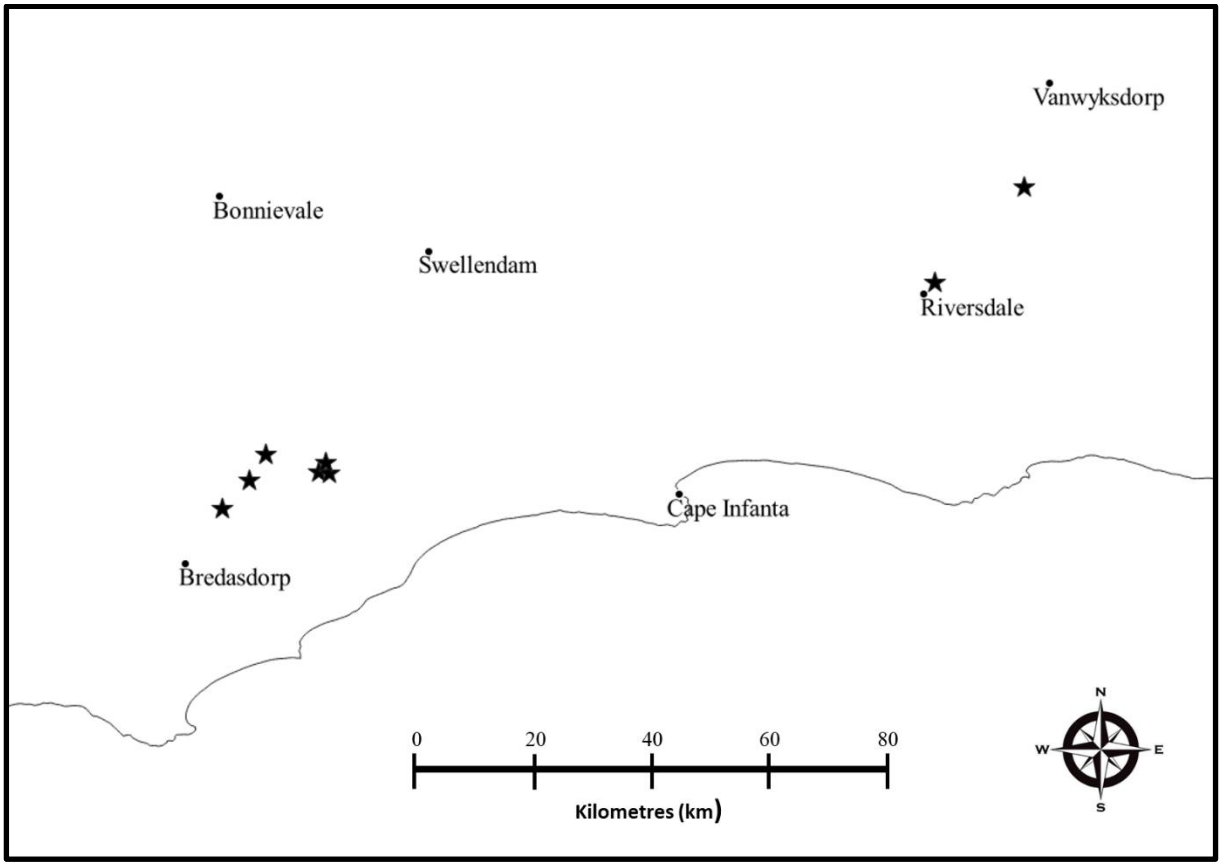

Figure 20: Distribution of *Polhillia connata*, black stars near Riversdale represent an approximation of Muir's collections that may be extinct.

Additional specimens examined

South Africa. WESTERN CAPE: **3321 (Ladismith):** Hills at Waterval in Klein Karoo, north of Langeberg (–CD), Sept 1925, *Muir 3745* (BOL, PRE). **3420 (Bredasdorp):** Rooivlei, Bredasdorp (–AC), 16 Oct 1982, *Bayer 3183* (NBG); Rooivlei farm, Swellendam area (–AC), 13 Oct 2003, *CREW 30* (NBG); 21 May 2017, *du Preez 287* (BOL); 15 Oct 2017, *du Preez 388* (BOL); Rooivlei farm, 0.5 km east of trig beacon 172 (–AC), 12 Sept 2000, *Helme 1752* (NBG); About 12 km NE Bredasdorp on Adoons Kop (–AC), 1 Aug 2001, *Helme 2062* (NBG); Adoonskop farm just off R319 (–AC), 15 Oct 2017, du Preez 390 (BOL); Adoonskop, N of Bredasdorp (–AC), 9 Oct 1986, *B.-E. van Wyk 2093* (JRAU), *2094* (JRAU); Overberg region, San Souci farm off R319 (–AC), *B.A. Walton 498* (NBG); Haasiesdrif farm, just off R319 (–AC), 14 Oct 2017, *du Preez 383* (BOL); Rooivlei farm, 20 miles North of Bredasdorp (–AC), 1 Oct 2012, *Stirton 13721* (BOL); Beyersdal farm (–AD), 13 Oct 2017, *du Preez 383* (BOL); Beyersdal farm, above Zoutrivier (–AD), 12 Jul 2017, *du Preez 519* (BOL). **3421 (Riversdale):** Riversdale, Hills near Riversdale (–AB), Oct 1923, *Muir 2803* (BOL); Flats near Riversdale (–AB), *Muir 2817* (PRE).

7. Polhillia curtisiae C.H.Stirt. & Muasya, S Afr. J. Bot. 87: 106 (2013).

Type: South Africa, Western Cape, **3420 (Bredasdorp):** Bredasdorp Dist., Haarwegskloof farm, 12 Oct 2011, *Stirton, Muasya & Curtis 13361* (BOL, holotype!; NBG, isotype!)

Plant an erect, sometimes spreading, sparsely branching shrub up to 1.0 m tall, sprouting from woody rootstock. *Branches* greyish-brown, young branches densely sericeous, becoming glabrescent with age, lenticels prominent. *Stipules* 2.0–4.0 mm long, deltoid, bifurcate, fused, sheathing stem, sericeous. *Leaves* digitately trifoliolate, pale green-grey, subsessile, shaggy pubescent; leaflets conduplicate, flattening partially during wet season, elliptic-oblanceolate, margin entire, apex acute, base cuneate; terminal leaflet 3.5–7.0 × 1.0–2.0 mm; lateral leaflets symmetrical, 3.0–7.0 × 1.0–2.0 mm. *Inflorescence* terminal axillary cluster of 1–2 flowers, subsessile; pedicel up to 3.0 mm long; bract single at base of pedicel, 1.0–1.5 mm long, linear, sericeous, caducous; ebracteolate. *Flowers* yellow, 10.0–12.0 mm long. *Calyx* campanulate, 4.0–5.0 mm long, bilabiate, yellow-green sometimes flushed maroon, villous; tube 3.0–3.5 mm long; dorsal lip bifid, teeth broadly triangular, ± 1.5 mm long, fused to ± half of lobe length; ventral lip trifid, teeth narrowly triangular, ± 1.5 mm long, fused for less than two thirds their length, apex acute-apiculate; inner tips of teeth

pubescent; *Standard petal* (9.0–) 11.0–13.5 mm long; claw linear, 3.0–4.5 mm long; lamina broadly ovate, 8.0–11.0 × 9.0–14.0 mm, apex emarginate, base cordate, back of standard sericeous over entire surface, except along margin. *Wing petals* 9.0–11.0 mm long, longer than keel; claw 3.0–4.0 mm long; lamina oblong-obovate, 7.0–9.0 × 3.5–5.0 mm, apex obtuse, base auriculate; rectangular and lunulate depression with lamellate sculpturing dorsi-basal above midline; pilose along apical half of midline. *Keel petals* 8.0–10.5 mm long; claw 3.0–4.0 (–4.8) mm long; lamina obtuse-lunate, 6.0–8.0 × 3.5–4.0 mm, apex obtuse, base triangular-auriculate, pilose along ventral front half of lamina, pocket present, basal along midline. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long, basifixed anthers, 1.1–1.2 mm long, and one carinal anther, 0.9–1.0 mm long, and five short, dorsifixed anthers, 0.7–0.8 mm long; filaments fused forming staminal tube, 5.9–7.3 mm long, slit dorsal, unfused portion remaining equidistant, sub-erect, neatly arranged around style. *Pistil* 9.0–11.0 mm long, subsessile; ovary narrowly oblong, 4.8–6.8 mm long, 0.9–1.0 mm high, sericeous, ovules 9; style terete, sub-erect, usually at about 45–60° angle, height of curvature 1.2–2.9 mm, glabrous except basally; stigma regular, capitate. *Pod* indehiscent, oblong, flattened, laterally compressed between seeds, tapering toward the apex and base, woody, densely silver shaggy hairy-velutinous, grey-brown, 17.0–27.0 × 3.0–4.0 mm. Seed ovate-reniform, testa olive-green to black-brown, smooth, 2.0–2.5 × 1.8–2.3 mm, 1.6–2.0 mm thick; hilum round, recessed, sub-terminal; rim aril inconspicuous; microphyll hidden. Pollen tricolpate; oblong to ovate in equatorial view, rounded-triangular in polar view, polar apices rounded; roughly 25.0–30.0 × 18.0–21.0 µm in size; semi-tectate, tectum micro-reticulate, lumina heterobrochaete, rounded; pollen kit ample; intra-luminary bacules present.

Flowering time: (February) April to August

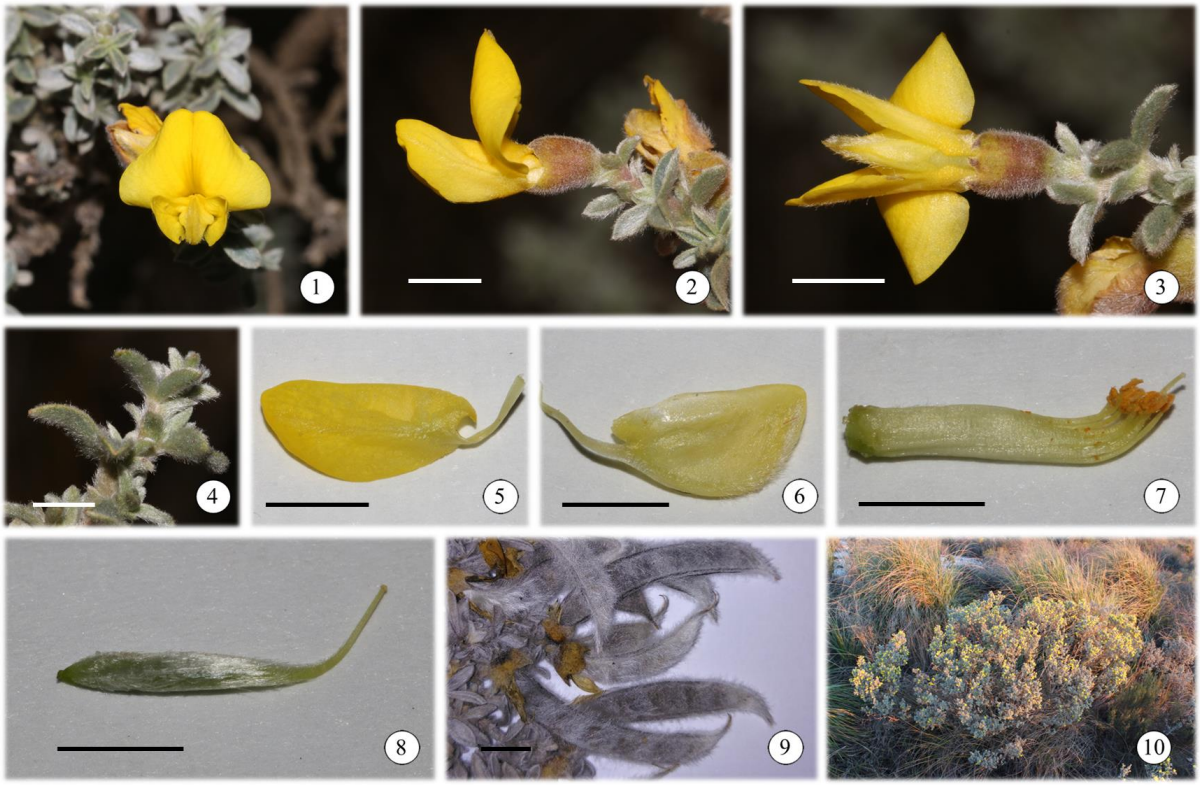

Figure 21: Composite plate of *Polhillia curtisiae*. 1 – Flower front view; 2 – Flower side view; 3 – Flower bottom view; 4 – Close-up of leaves and stipules; 5 – Wing petal; 6 – Keel petal; 7 – Androecium; 8 – Gynoecium; 9 – Pods; 10 – Habit. Scale bars: 4 mm. Photographs by B. du Preez.

Etymology

The specific epithet "curtisiae" is in honour of Dr Odette Curtis who has studied plants in the Overberg Renosterveld for over a decade, finding numerous species new to science and playing an integral role in the conservation of Renosterveld vegetation fragments in this region.

Diagnostic characters

Polhillia curtisiae (Figure 21) is unlikely to be confused with any other species in its immediate surrounding area in the Overberg. It is morphologically most similar to *Polhillia xairuensis*, as both species have shaggy hairy leaves (*versus* adpressed sericeous-canescent leaves in other Overberg species); unfused parts of stamens spreading, loosely arranged around style (*versus* unfused parts of stamens tightly arranged around style in other Overberg species); and style sub-erect, usually angled at between 45–60° (*versus* style erect, usually angled at between 70–90° in other Overberg species). Confusion between this species and *P. xairuensis* is unlikely as they occur on opposite side of the Breede River. Furthermore, *P.*

curtisiae is an erect shrub of 0.5–1.0 m tall, while *P. xairuensis* is a taller erect shrub of up to 1.8 m tall. In *P. curtisiae* unfused part of the stamens remain equidistant in spreading, and are neatly arranged around style, while the unfused parts of stamens are loosely spreading and unevenly arranged around the style in *P. xairuensis*. Petal characters provided the final differences between *P. curtisiae* and *P. xairuensis.* In the former species the wing petals are oblong-obovate and the keel petal apices are obtuse and rounded, while the wing petals of the latter are strongly obliquely oblong and the keel petal apices are acute and cultrate.

Distribution, ecology and conservation status

Polhillia curtisiae is endemic to the Haarwegskloof and Spitskop area of the southern Overberg district between Swellendam and Bredasdorp (Figure 22). This species was only known only from the type locality at the start of 2017, but is now known from 4 scattered locations in this area. The known meta-population of this species has thus now increased from roughly 60 plants to about 500. Populations of this species are fragmented and much of its historical habitat has been lost to agriculture. Remaining sub-populations are in most cases small, but are not threatened by further agricultural expansion as these remaining populations are located on rocky outcrops. Sub-population sizes are, however, of great concern as most of the sites contain less than 50 mature individuals, one of which is limited to a single plant. The type locality of this species comprises of Eastern Rûens Shale Renosterveld (FRs 13) on grey kaolinite clay on Haarwegskloof Renosterveld Reserve, while others locations were found growing on ferricrete and silcrete outcrops where plants grow in Rûens Silcrete Renosterveld (FRc 2) (Mucina and Rutherford, 2006). This species appears to favour northerly slopes and grows either on hill tops or gentle to moderate slopes. The Red Data List status of this species is assessed as EN A2c; B1ab(i,ii,iii,iv,v)+B2ab(i,ii,iii,iv,v) following the latest IUCN categories and criteria (IUCN, 2012).

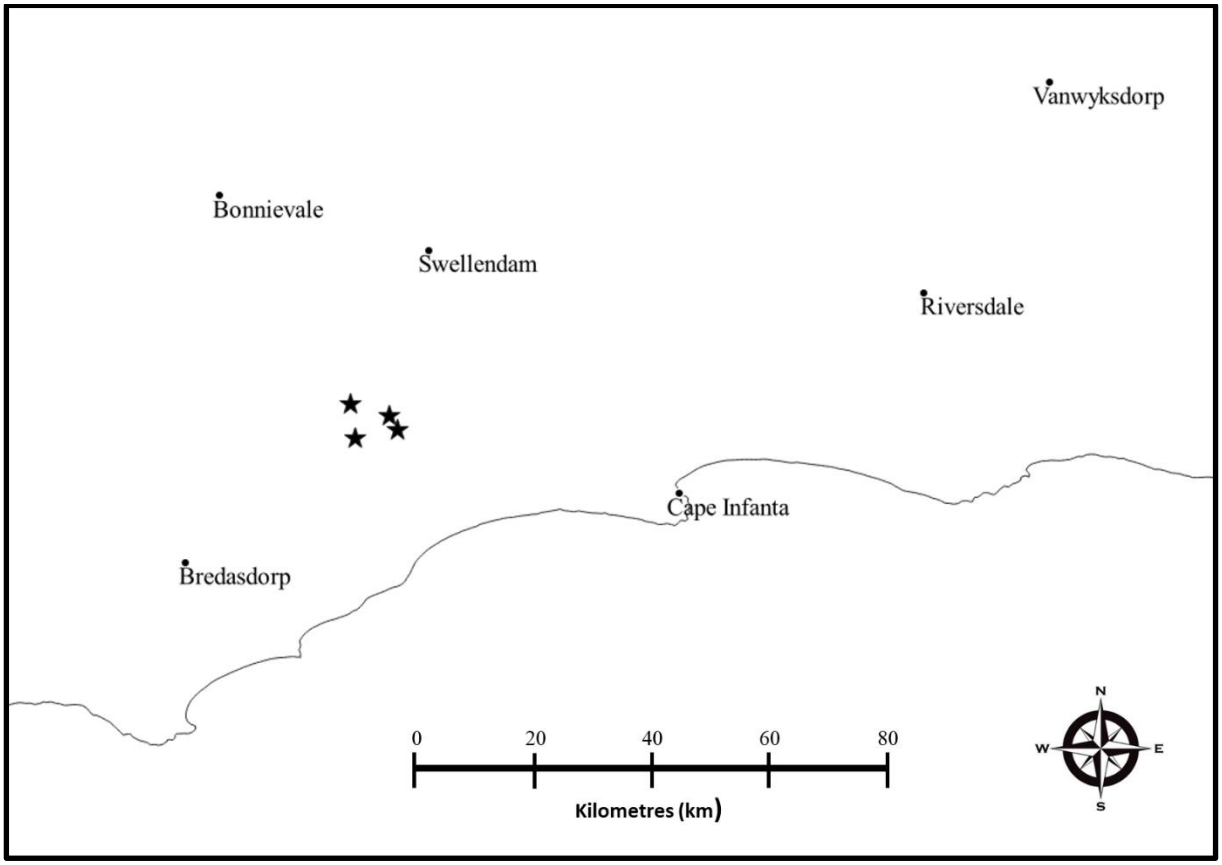

Figure 22: Distribution of *Polhillia curtisiae*.

Additional specimens examined

South Africa. WESTERN CAPE: **3420 (Bredasdorp):** About 35km NE of Bredasdorp, Haarwegskloof 52 (–AD), 16 Aug 2008, *Helme & Curtis 5562* (BOL, NBG); Spitskop (east) farm on hill above gums (–AD), 20 May 2017, *du Preez 282* (BOL); Spitskop North, koppie east of road (–AD), 9 Jul 2018, *du Preez 510* (BOL, NBG, PRE); Koppie north of Haarwegskloof research centre (–AD), 20 May 2017, *du Preez 286* (BOL), 9 Jul 2018, *du Preez 511* (BOL); 6 Aug 2017, *du Preez 307* (BOL); Haarwegskloof farm (–AD), 2 Dec 2011, *Stirton & Curtis 13461* (BOL); 12 Oct 2011, *Stirton & Curtis 13363* (BOL); 12 Oct 2011, *Stirton 13361* (BOL); Muurkraal farm on small koppie (–AD), 9 Sept 2017, *du Preez 334* (BOL).

8. Polhillia xairuensis B.du Preez, *sp. nov*.

Type: South Africa, Western Cape, **3420 (Bredasdorp):** Suurbraak Region, Voorstekop farm (–BC), 11 Aug 2017, *du Preez 315* (BOL, holotype!; K, NBG, MO, isotype!)

Plant an erect, rounded shrub up to 1.0–1.5 (–1.8) m tall, often wider than tall, sprouting from woody rootstock. *Branches* greyish-brown, glabrescent, terminal burst branching present, often forming a closed, dense, umbrella-like canopy; lenticels prominent on old stems. *Stipules* 1.5–2.5 mm long, deltoid, bifurcate, fused, sheathing stem, sericeous. *Leaves* digitately trifoliolate, densely shaggy hairy, subsessile; leaflets conduplicate, flattening partially during the wet season, lanceolate to narrowly-oblong, apex acute-apiculate, base cuneate, margins entire; terminal leaflet 3.0–5.5 × 1.0–2.0 mm; lateral leaflets symmetrical, 2.5–5.0 × 1.0–2.0 mm. *Inflorescence* terminal axillary cluster of two, sometimes one flower(s), subsessile; pedicel up to 3.5 mm long; bract single at base of pedicel, 1.0–1.5 mm long, linear, sericeous, caducous; ebracteolate. *Flowers* yellow, 10.0–11.5 mm long. *Calyx* campanulate, 3.5–5.0 mm long, bilabiate, green, often flushed maroon, villous; tube 2.5–3.5 mm long; dorsal lip bifid, teeth broadly triangular, 1.0–1.5 mm long, fused for less than one-third their length, tips acute; ventral lip trifid, teeth narrowly-triangular, 1.0–1.5 mm long, fused for two-thirds their length to almost entirely fused, tips variable, apiculate-rounded; inner tips of teeth pubescent. *Standard petal* 11.0–12.5 mm long; claw linear, 4.0–4.5 mm long; lamina broadly ovate, 7.0–8.5 × 9.5–11.0 mm, apex emarginate, base cordate; back of standard sericeous except along margin. *Wing petals* 9.0–11.0 mm long, equal to or slightly longer than keel; claw 3.5–4 mm long; lamina obliquely oblong, 8.0–9.0 × 3.5–4.0 mm, apex rounded, base broadly auriculate, basal dorsal margin involute, long elliptical depression and lamellate sculpturing dorsi-basal above midline; glabrous to rarely sparsely pubescent along apical half of midline. *Keel petals* 8.5–10.0 mm long; claw 4.0–4.5 mm long; lamina lunate, 7.5–8.0 × 3.0–4.0 mm, apex acute, cultrate, base auriculate, pilose along front edge; pocket present, basal along midline. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long, basifixed anthers, 1.3–1.4 mm long, and one carinal anther, 1.2 mm long, and five short, dorsifixed anthers, 0.9–1.0 mm long; filaments fused forming staminal tube, 7.4–8.2 mm long, slit dorsal, unfused portion spreading, loosely arranged around style. *Pistil* 10.0–11.3 mm long, subsessile; ovary narrowly oblong, 6.2–8.2 mm long, 0.9–1.2 mm wide, sericeous, ovules 9; style terete, sub-erect, usually at about 45° angle, height of curvature 1.1–1.9 mm, glabrous except basally; stigma regular, capitate. *Pod* indehiscent, oblong, non-plicate, laterally compressed between seeds, straight to slightly falcate, tapering toward the

apex and base, fibrous, sericeous, olive-green, 21.0–28.0 × 3.0–3.5 mm. *Seed* ovate-reniform; testa dark black-brown, smooth, 2.2–3.0 × 1.9–2.4 mm, 1.4–1.7 mm thick; hilum round, recessed, sub-terminal; rim aril inconspicuous; microphyll hidden. *Pollen* tricolpate, oblong in equatorial view, rounded-triangular in polar view, polar apices truncate-rounded, roughly 27.0–31.0 ×16.0–18.0 µm in size, semi-tectate, tectum micro-reticulate to coarsely-reticulate, lumina heterobrochaete, rounded to channelled, pollen kit ample, intra-luminary bacules present.

Flowering time: July to September

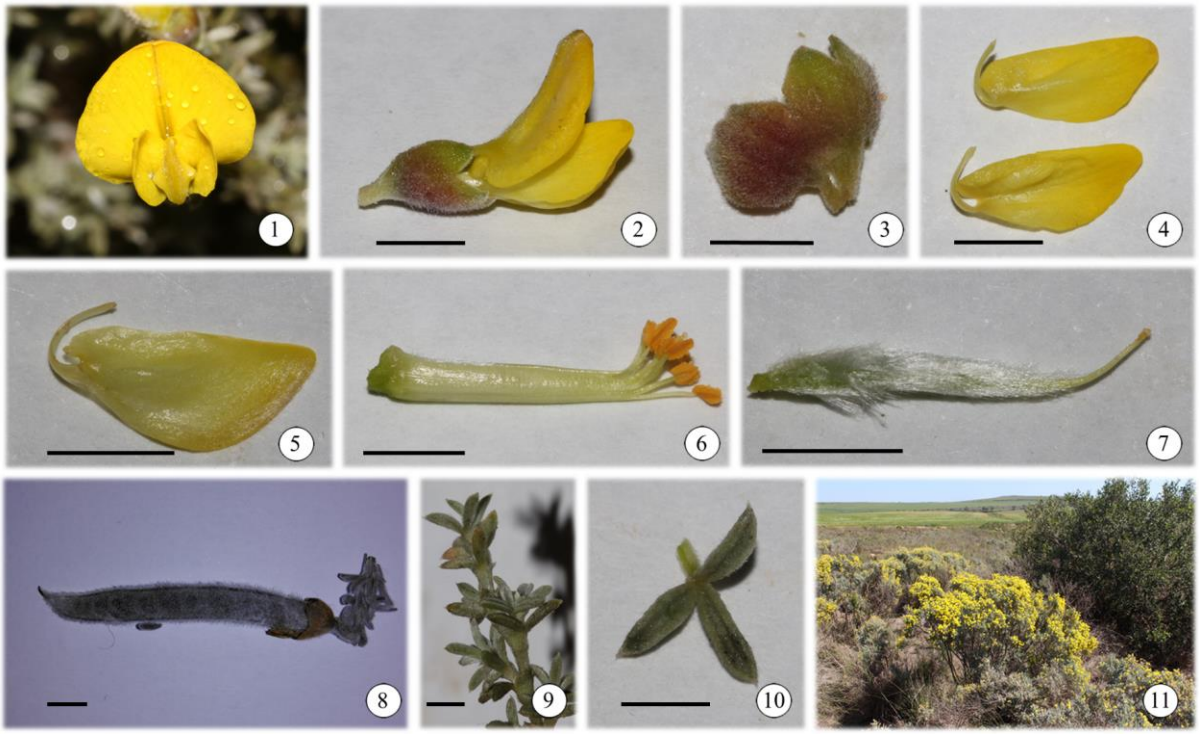

Figure 23: Composite plate of *Polhillia xairuensis*. 1 – Flower front view; 2 – Flower side view; 3 – Calyx; 4 – Wing petals; 5 – Keel petal; 6 – Androecium; 7 – Gynoecium; 8 – Pod; 9 – Leaves and stipules; 10 – Close-up of leaf; 11 – Habit. Scale bars: 4 mm. Photographs by B. du Preez.

Etymology

The specific epithet is derived from the Khoisan name for the Suurbraak area, namely "!Xairu" which means 'Beauty' or 'A place called Paradise', referring to the beautiful nature of the area (Fourie, 2013). Although much of the area has now been transformed for agriculture, the Langeberg Mountains above the town Suurbraak remain a beautiful sight and lowland vegetation fragments hold beautiful floral gems such as this *Polhillia* species.

Diagnostic characters

Polhillia xairuensis (Figure 23) was previously thought to be a variant of *Polhillia pallens*, but is instead morphologically most similar to *P. curtisiae* that occurs further west. It may be confused with *P. pallens*, as there are populations in close proximity on the eastern side of the Breede River. It can be distinguished from *P. pallens* by having densely shaggy hairy leaves (*versus* adpressed sericeous-canescent leaves); leaves lanceolate to narrowly oblong (*versus* elliptical to broadly-oblong); stamens loosely spreading around style, style at ± 45° angle (*versus* stamens tightly arranged, erect around style, style at 70–90° degree); plants with terminal burst branching often forming dense closed canopies (*versus* plants with scattered branching and no closed canopy); wing petals completely glabrous or very sparsely pubescent (*versus* wings clearly pubescent along midline); keel petal apex acute (*versus* keel petal apex obtuse-rounded); lower three calyx teeth almost completely fused with rounded-obtuse tips (*versus* lower three calyx teeth fused for less than half their length with acute tips). Confusion between this species and *P. curtisiae* is unlikely, as they occur on opposite sides of the Breede River and can be distinguished by plants erect up to 1.8 m tall (*versus* erect shrub less than 0.5–1 m tall); unfused part of stamens loosely spreading unevenly around style (*versus* unfused part of stamens remaining equidistant in spreading, neatly arranged around style); wing petals strongly obliquely oblong (*versus* wing petals oblong-obovate); keel petal apex acute, cultrate (*versus* keel petal apex obtuse, rounded).

Distribution, ecology and conservation status

Polhillia xairuensis is restricted to a few sites in Eastern Rûens Silcrete Renosterveld (FRc 2) in the Suurbraak area (Figure 24) between Swellendam and Heidelberg (Mucina and Rutherford, 2006). It grows in loamy soils, usually in gullies or on gentle slopes. Much of this species' historical habitat has been transformed for agriculture, but roughly 1000 plants persist on small vegetation fragments that are unlikely to be ploughed in future. The Red Data List status of this species is assessed as EN A2c; B1ab(iii,v)+B2ab(iii,v) following the latest IUCN categories and criteria (IUCN, 2012).

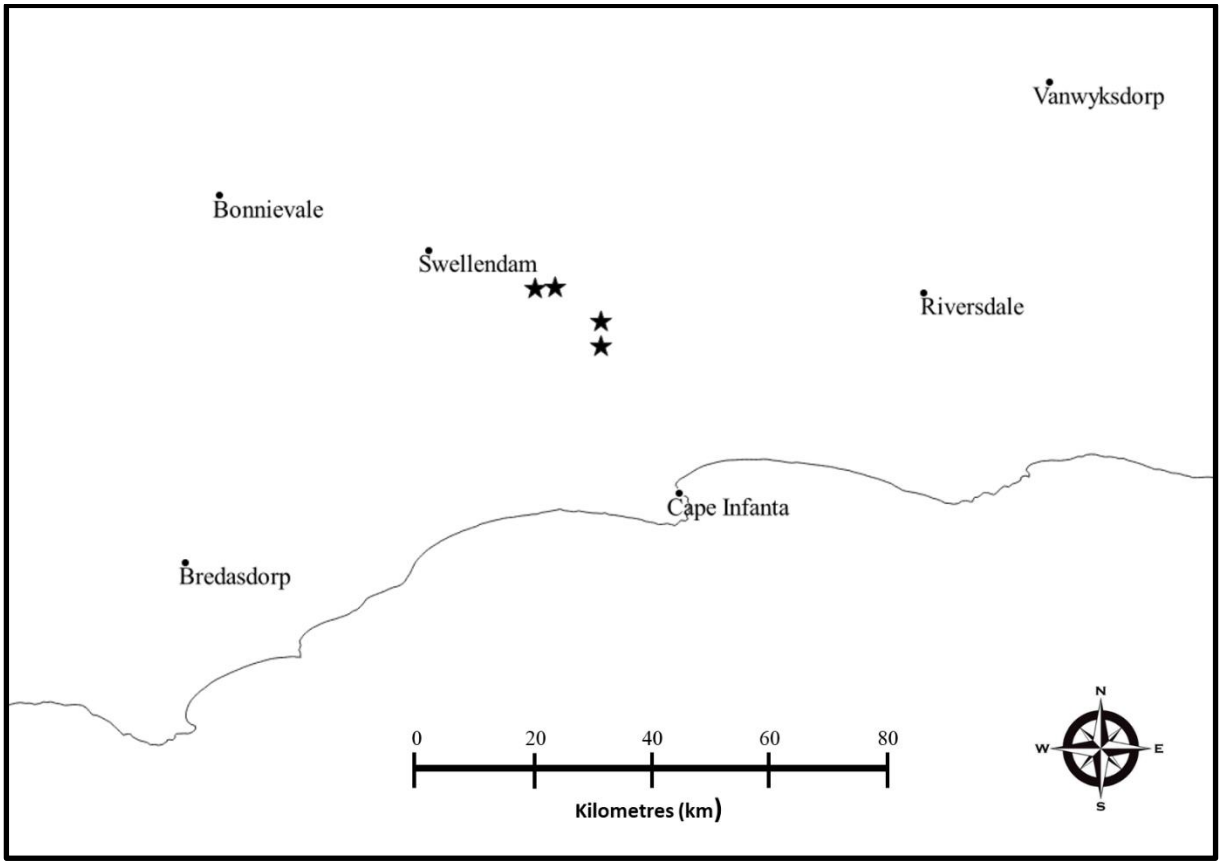

Figure 24: Distribution of *Polhillia xairuensis*.

Additional specimens

South Africa. WESTERN CAPE: **3420 (Bredasdorp):** Swellendam, 15 km SE of Buffeljagsrivier on Hartebeesterivier 278 (–BA), 27 Sept 2001, *Helme 2282* (NBG); Niekerkshek, 20 km E of Swellendam (–BA), 19 Jul 2003, *Kloppers s.n. sub 195427* (NBG); Voorstekop farm, between Heidelberg and Swellendam (–BA), 11 Aug 2017, *du Preez 315* (BOL, K, MO, NBG); 25 Nov 2011, *Muasya, Stirton & Curtis 6265* (BOL); Voorstekop farm, near guest lodge (–BA), 10 Sept 2017, *du Preez 335* (BOL); Suurbraak commonage, Alan Jafta's farm (–BA), 10 Sept 2017, *du Preez 337* (BOL, NBG); 25 Nov 2011; *Stirton & Curtis 13531* (BOL); Zoutkloof farm, S of Voorstekop (–BA), 16 Oct 2017, *du Preez 392* (BOL); Oudekraalskop (–BA); 19 Jun 2012; *Curtis 111* (BOL); Suurbraak (–BA), 10 Sept 2011, *Curtis 14* (BOL).

9. Polhillia pallens C.H.Stirt. S Afr. J. Bot. 52: 2 (1986). Type: South Africa, Western Cape, **3420 (Bredasdorp):** 3km west of Breede River mouth on S side of river along access road to Kontiki (–BD), 22 Feb 1981, *C.J. Burgers 2633* (NBG, holotype!; K, isotype!)

Erect shrub up to 1.5–2.5 m tall, loosely branching, sprouting from woody rootstock. *Branches* greyish-brown, glabrescent, lenticels prominent. *Stipules* 1.5–3.0 mm long, deltoid, bifurcate, fused, sheathing stem, sericeous. *Leaves* digitately trifoliolate, sericeous, subsessile, leaflets flattened to partially conduplicate, elliptical-oblong, margin entire, involute, terminal leaflet 3.5–7.0 × 1.0–3.0 mm, lateral leaflets symmetrical, 3.0–6.5 × 1.0–3.0 mm, apex acute-obtuse, base acute-attenuate. *Inflorescence* terminal axillary cluster of 1–2 flowers, subsessile; pedicel up to 2.0 mm long; bract single at base of pedicel, linear, sericeous, 1.0 mm long, caducous; ebracteolate. *Flowers* yellow, 10.0–11.5 mm long. *Calyx* campanulate, 4.0–4.5 mm long, bilabiate, yellow-green, sometimes flushed maroon, villous; tube 2.5–3.0 mm long; dorsal lip bifid, teeth broadly triangular, teeth 1.5–2.0 mm long, fused for less than one third of their length, tips acute-obtuse; ventral lip trifid, triangular, teeth 1.5–2.0 mm long, fused to about two thirds the lobe length, tips acute, inner tips of teeth pubescent. *Standard petal* 10.0–12.0 mm long, claw linear, 3.0–4.0 mm long, lamina broadly ovate, 8.0–8.5 (–10.0) × 10.0–13.0 mm, apex emarginate, base cordate, back of standard sericeous, except along margin. *Wing petals* 9.5–11.0 mm long, equal to or slightly longer than keel; claw 3.2–4.0 mm long; lamina obliquely-oblong, 7.5–8.0 (–9.0) × 4.0–4.5 mm, pilose along midrib, apex rounded-truncate, base broadly auriculate, triangular and lunate depression with lamellate sculpturing dorsi-basal above midline. *Keel petals* 9.0–10.5 mm long, claw 3.5–4.5 mm long, lamina obliquely-oblong, 7.0–8.0 × 3.8–4.5 mm, pilose along anterior half, apex rounded, base triangular-auriculate, pocket present, basal along midline. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long, basifixed anthers, 1.2–1.3 mm long, and one carinal anther, 1.1 mm long, and five short, dorsifixed anthers, 0.8 mm long; filaments fused forming staminal tube, 6.7–8.5 mm long, slit dorsal, unfused portion remaining closely united, rostrate, tightly arranged around style. *Pistil* 8.2–10.2 mm long, subsessile; ovary narrowly oblong, 4.6–6.3 mm long, 0.9–1.2 mm wide, sericeous, ovules 9; style terete, erect, usually at 70–90° angle, height of curvature 2.7–3.6 mm, glabrous, except basally; stigma regular, capitate. *Pod* indehiscent, oblong, non-plicate, laterally compressed between seeds, straight, tapering toward the apex and base, fibrous, sericeous, olive green, 18.0–30.0 × 3.0–3.5 mm. *Seed* ovate-reniform, testa olive-green to dark-brown, smooth, 2.0–2.9 × 1.8–2.2 mm, 1.6–1.9 mm thick; hilum round, recessed, sub-

terminal; rim aril inconspicuous; microphyll hidden. *Pollen* tricolpate, oblong to ovate in equatorial view, rounded-triangular in polar view, polar apices truncate-rounded, roughly 22.0–32.0 × 14.0–20.0 µm in size, semi-tectate, tectum micro-reticulate to finely-reticulate, lumina heterobrochaete, rounded to oblong, pollen kit ample, intra-luminary bacules present.

Flowering time: (Feb) May to August

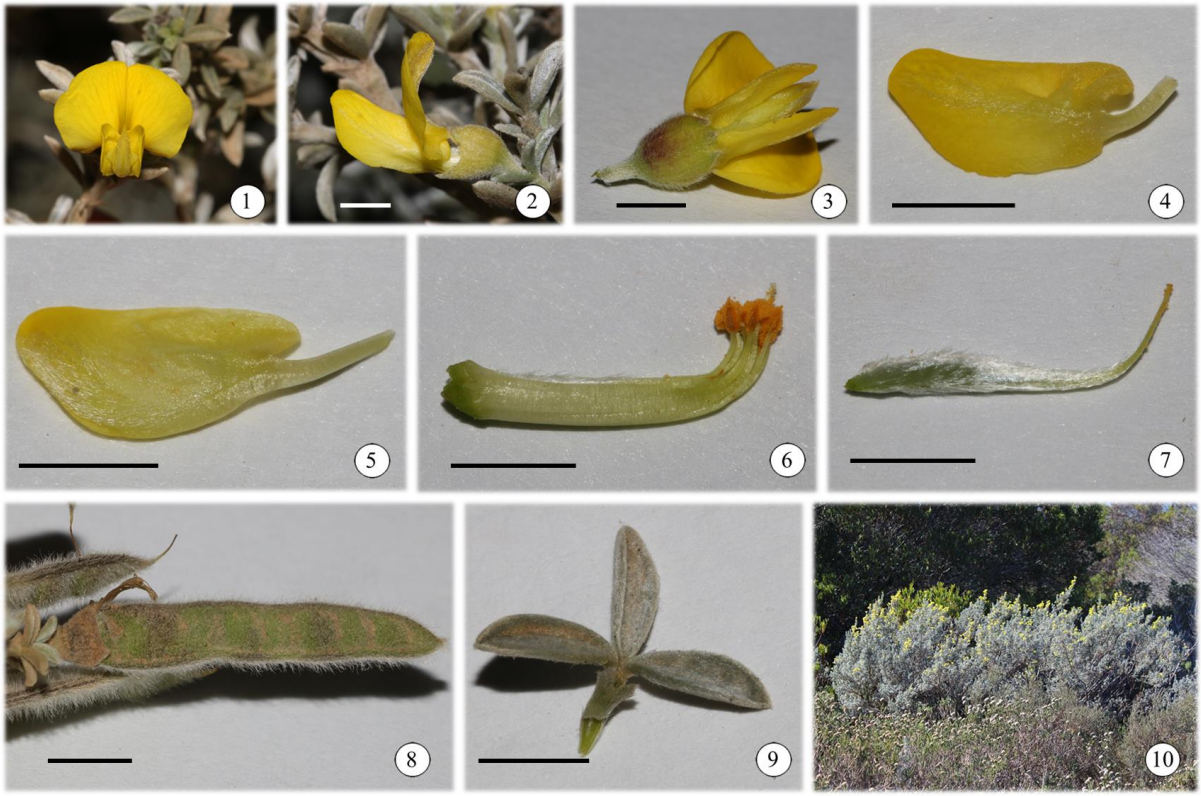

Figure 25: Composite plate of *Polhillia pallens*. 1 – Flower front view; 2 – Flower side view; 3 – Flower bottom view; 4 – Wing petal; 5 – Keel petal; 6 – Androecium; 7 – Gynoecium; 8 – Pod; 9 – Close-up of leaf and stipules; 10 – Habit. Scale bars: 4 mm. Photographs by B. du Preez.

Etymology

The specific epithet “pallens” refers to the pale silvery leaves of this species.

Diagnostic characters

Polhillia pallens (Figure 25) is morphologically variable, especially in terms of the shape of the wing and keel petals, but not to an extent that would warrant further splitting. It is tricky to distinguish between this species and *P. stirtoniana*, but it has straight, elliptical-oblong leaflets, 1.0–3.0 mm wide (*versus* arcuate, linear to narrowly-lanceolate leaflets, less than 1 mm wide); and it is a tall, sparsely branched shrub of up to 2.5 m tall (*versus* erect, rounded shrub with dense terminal burst branching up to 1.0 m tall). It can also be distinguished from other similar species in the area such as *P. curtisiae* and *P. xairuensis* by having adpressed sericeous-canescent, grey leaves (*versus* densely shaggy hairy, blue-green leaves); unfused parts of stamens remaining closely united around the style, style erect at 70–90º angle (*versus* unfused parts of stamens loosely spread around style, style sub-erect at 45–60º angle); and by being a tall, sparsely branched shrub of up to 2.5 m tall (*versus* erect, rounded shrub with dense terminal burst branching, 0.5–1.0 m or 1.0–1.8 m tall, respectively).

Distribution, ecology and conservation status

This species was previously broadly circumscribed to include both *Polhillia stirtoniana* and *P. xairuensis*. It is restricted to only a few sites along and near the lower Breede River region (Figure 26), where it grows in clay soil in Eastern Rûens Shale Renosterveld (FRs 13) and in transitional vegetation with Overberg Dune Strandveld (FS 7) on the southern banks of the Breede River (Mucina and Rutherford, 2006). This species is threatened by continued agricultural expansion, habitat fragmentation and by severe alien species encroachment at the type locality. Some populations are, however, still healthy and plants grow abundantly in at least three of the known sites. The Red Data List status of this species is assessed as VU A2c; B1ab(iii,iv,v)+B2ab(iii,iv,v) following the latest IUCN categories and criteria (IUCN, 2012).

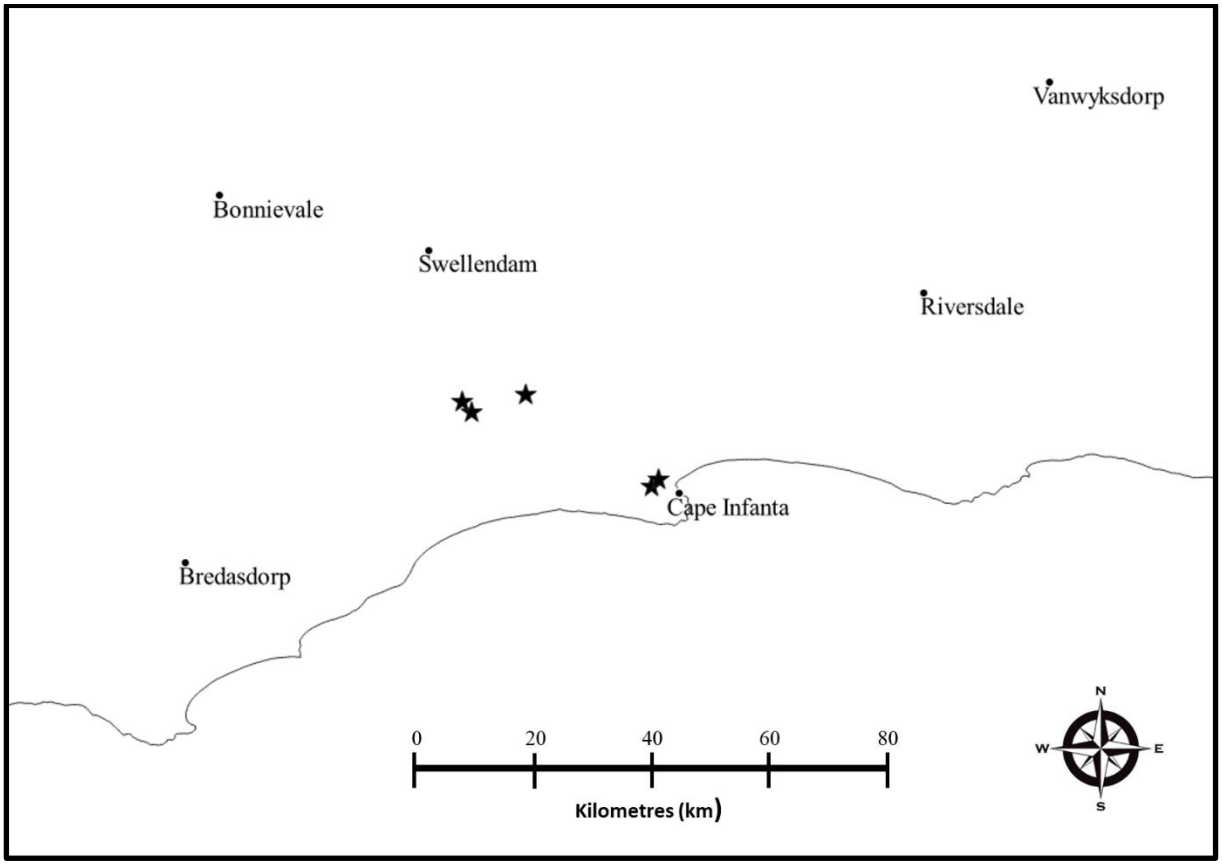

Figure 26: Distribution of *Polhillia pallens*.

Additional specimens examined

South Africa. WESTERN CAPE: **3420 (Bredasdorp):** 18 km. S by E of Buffeljachtsrivier (–BC), 21 Jun 1968, Acocks 23992 (NBG); Road heading due north of Malgas, east of Breede River (–BC), 3 Jun 2017, *du Preez 289* (BOL), *291* (BOL, NBG), 10 Jul 2018, *du Preez 514* (BOL); Napkysmond, near Now-I-Know farm (–BC), 11 Aug 2017, *du Preez 319* (BOL); 3 km west of Breede River mouth on S side of river along access road to Kontiki (–BD), 22 Feb 1981, *C.J. Burgers 2633 Ex hort* (NBG); 8 Aug 2017, *du Preez 310* (BOL, NBG); On slope, above and west, of harbour at Witsand (–BD), 16 Feb 2005, *Euston-Brown 700* (NBG); Kloof NW of Mudlark Lodge (–BD), 10 Jul 2018, *du Preez 512* (BOL).

10. Polhillia stirtoniana B.du Preez, *sp. nov*.

Type: South Africa, Western Cape, **3420 (Bredasdorp):** Bredasdorp Region, Plaatjieskraal farm, farm road leading west (–AD), 7 Aug 2017, *du Preez 309* (BOL, holotype!; K, isotype!)

Plant an erect, rounded shrub up to 0.8–1.2 m tall, often more wide than tall, sprouting from woody rootstock. *Branches* greyish-brown, glabrescent, terminal burst branching present, forming a closed, dense, umbrella-like canopy when mature; lenticels prominent. *Stipules* 1.5–2.5 mm long, deltoid, fused, sheathing stem, sericeous. *Leaves* digitately trifoliolate, subsessile, sericeous; leaflets conduplicate, tightly closed during dry season, opening slightly in wet season; narrowly linear-lanceolate, arcuate, apex acute, base cuneate, margins entire; terminal leaflet 3.0–5.5 × 0.5–0.7 (–1.0) mm; lateral leaflets symmetrical, 2.5–5.5 × 0.5–0.7 (–1.0) mm. *Inflorescence* single (rarely two) terminal axillary flowers, subsessile; pedicel up to 3.0 mm long; bract linear, sericeous, ± 1.0 mm long, caducous, ebracteolate. *Flowers* bright yellow, 9.0–11.0 mm long. *Calyx* 3.0–4.0 mm, villous, bilabiate; tube 2.0–2.5 mm, dorsal lip bifid, fused for less than one third of lobe length, teeth 1.0–1.5 mm, triangular, ventral lip trifid, fused for about two thirds of lobe length, teeth 1.0–1.5 mm, triangular, tips acute. *Standard petal* 10.0–12.5 mm long; claw linear, 3.0–4.0 mm long, lamina broadly ovate, 8.0–11.0 × 10.0–13.5 mm, apex emarginate, base cordate, adaxial surface glabrous, abaxial surface sericeous, glabrous only along edge. *Wing petals* 9.0–11.0 mm long, equal to slightly longer than keel, claw 3.0–4.0 mm long, lamina obliquely oblong, 8.0–9.0 × 4.0–4.8 mm, apex truncate, base rounded auriculate, shortly pilose along midrib to glabrous, lunulate and rectangular depression with lamellate sculpturing above midrib. *Keel petals* 8.0–10.0 mm long, claw 3.5–5.0 mm long, lamina oblong-elliptical, 6.5–7.5 × 3.5–4.5 mm, shortly pilose along front edge, pocket present along posterior end of midrib. *Stamens* 10, monodelphous; anthers dimorphic, alternating between four long, basifixed anthers, 1.3–1.4 mm long, and one carinal anther, 1.0–1.2 mm long, and five short, dorsifixed anthers, 0.7–0.8 mm long; filaments fused forming staminal tube, 6.9–8.0 mm long, slit dorsal, unfused portion spreading, loosely arranged around style. *Pistil* 8.2–9.2 mm long, subsessile; ovary narrowly oblong, 5.7–6.3 mm long, 0.9–1.2 mm wide, sericeous, ovules 9–11; style terete, sub-erect, usually at 70–80° angle, height of curvature 2.1–2.5 mm, glabrous, except basally; stigma regular, capitate. *Pod* indehiscent, oblong, laterally compressed between seeds, weakly plicate, straight to slightly falcate, tapering toward the apex and base, sericeous, pale olive-green, 17.0–29.0 × 3.0–4.0 mm. *Seed* ovate-reniform, testa olive-green to dark-brown,

smooth, 1.9–2.7 mm × 1.8–2.4 mm, 1.6–2.1 mm thick; hilum round, recessed, sub-terminal; rim aril inconspicuous; microphyll hidden. *Pollen* tricolpate, oblong to ovate in equatorial view, rounded-triangular in polar view, polar apices truncate-rounded, roughly 26.0–32.0 × 15.0–20.0 µm in size, semi-tectate, tectum micro-reticulate to finely-reticulate, lumina heterobrochaete, rounded to oblong, pollen kit ample, intra-luminary bacules present.

Flowering time: June–September

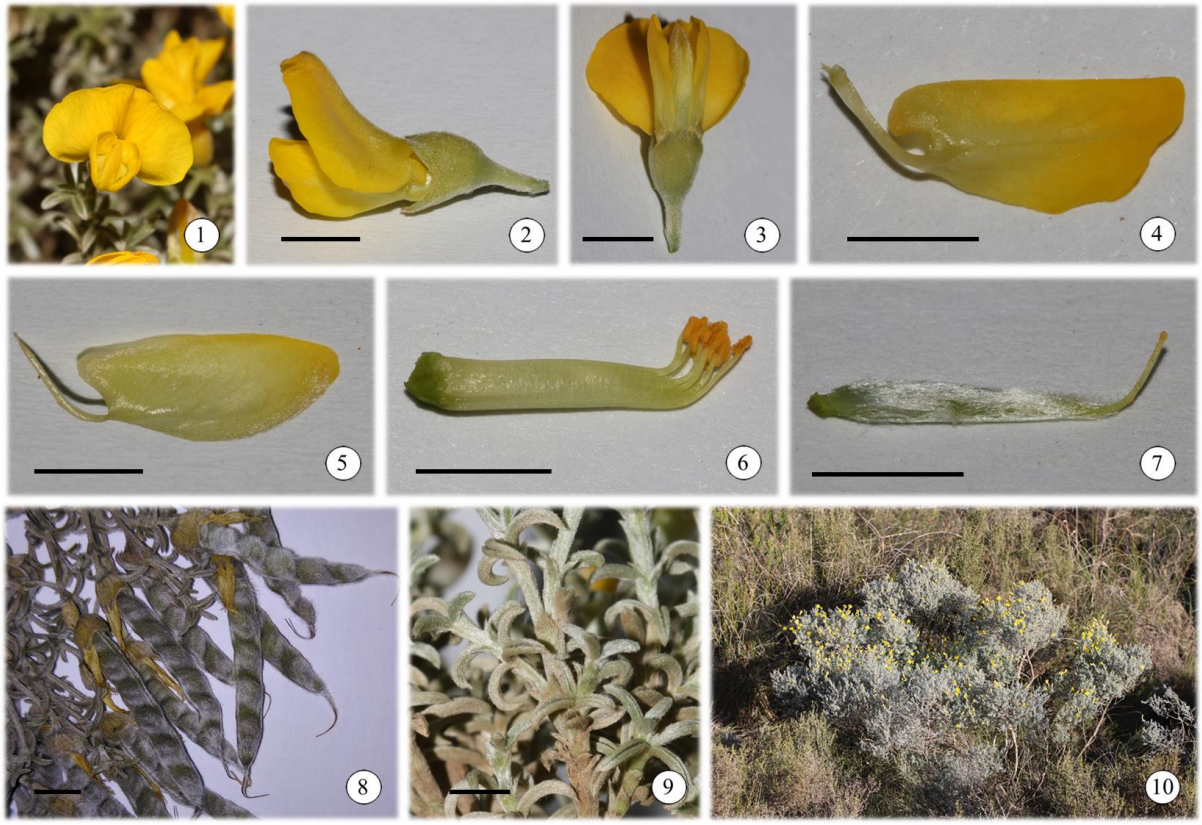

Figure 27: Composite plate of *Polhillia stirtoniana*. 1 – Flower front view; 2 – Flower side view; 3 – Flower bottom view; 4 – Wing petal; 5 – Keel petal; 6 – Androecium; 7 – Gynoecium; 8 – Pods; 9 – Close-up of leaves and stipules; 10 – Habit. Scale bars: 4 mm. Photographs by B. du Preez.

Etymology

The specific epithet honours Prof. Charles Howard Stirton for his extensive work on Cape legumes and his late wife, Dr Jana Stirton (Born Žantovská) who shared his love and passion for the diversity and conservation of the Overberg Renosterveld. They assisted in the formation of the Overberg Renosterveld Conservation Trust and establishment of the Haarwegskloof Private Nature Reserve and research centre. Prof. Stirton has especially mentored me in my development as botanist.

Diagnostic characters

Polhillia stirtoniana (Figure 27) was previously circumscribed as part of *Polhillia pallens*, but is distinguished from other Overberg species by its very narrow, arcuate leaves that are not wider than 1.0 mm (*versus* at least 1.0–5.0 mm in other species); dense terminal burst branching forming a closed, umbrella like canopy often wider than the plant height (*versus* shrub not forming closed canopy in other species, except *P. xairuensis*); and pods that are weakly plicate (*versus* pods not plicate in other species).

Distribution, ecology and conservation status

Polhillia stirtoniana often grows abundantly in sheltered gullies and cooler south facing slopes in Eastern and Central Rûens Shale Renosterveld (FRs 12 & FRs 13) (Mucina and Rutherford, 2006). The species is distributed from Potberg in the east to the R319 in the west with an outpost at Stormsvlei further north (Figure 28). It is the most abundant of all *Polhillia* species. This may be due to being protected from ploughing, as they prefer steep slopes and deep gullies and is often the dominant species in these habitats. The species, however, remains threatened by fragmentation throughout its distribution range and some sub-populations have been lost to recent ploughing. At present, this species is known from 12 extant locations, and more may be found as more areas of the Overberg are explored. The Red Data List status of this species is assessed as NT A2c; B1ab(iii,iv,v)+B2(iii,iv,v) following the latest IUCN categories and criteria (IUCN, 2012).

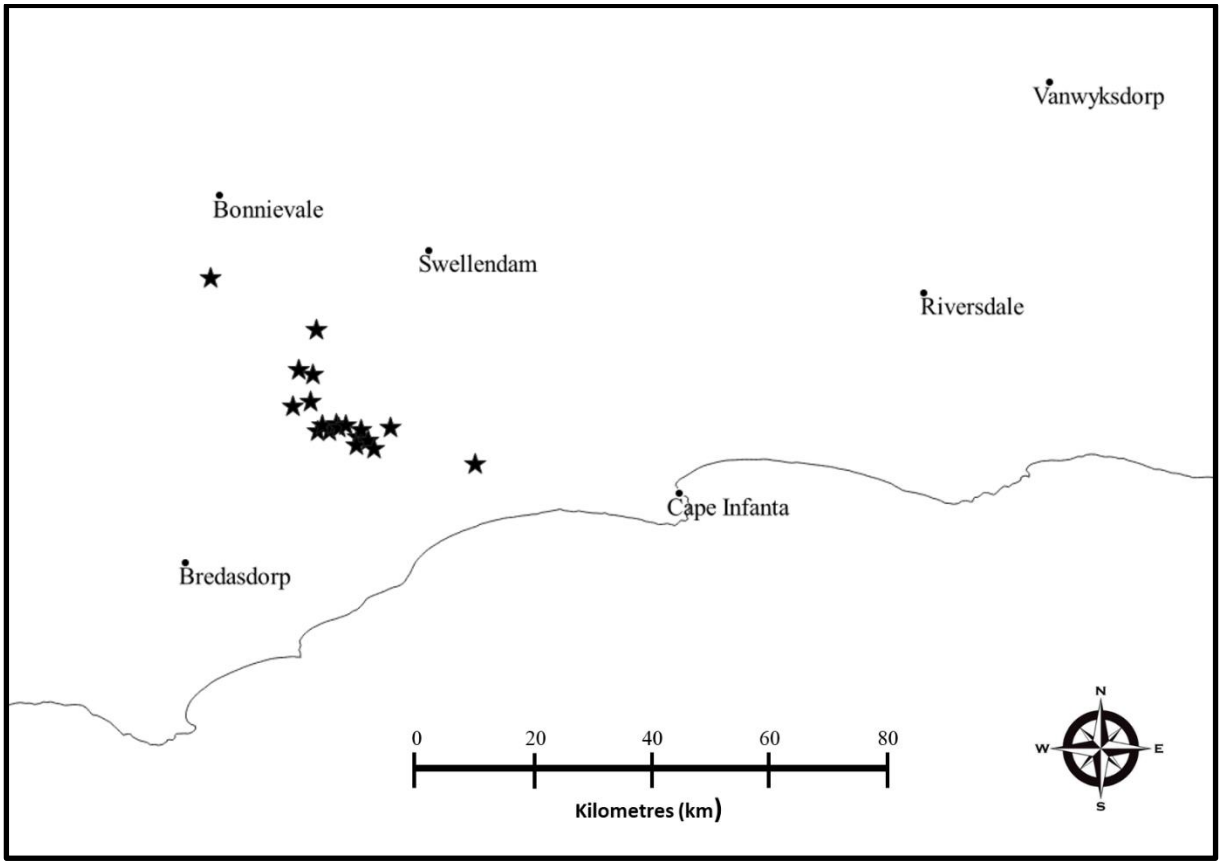

Figure 28: Distribution of *Polhillia stirtoniana*.

Additional specimens

3420 (Bredasdorp): Witkop, 15 km ESE of Stormsvlei (–AA), 1 Oct 1983, *C.J. Burgers 3212* (K, NBG); Top of Stormsvleikloof (–AA), 4 Jul 1987, *B.-E. van Wyk 2706* (JRAU); Farm Uitvlugt (–AC), 11 Jul 2018, *du Preez 517* (BOL); Koppies on farm Uitkyk (–AC), 11 Jul 2018, *du Preez 518* (BOL); Near Remhoogte, N of Bredasdorp (–AC), 9 Oct 1986, *B.-E. van Wyk 2095* (JRAU), *2096* (JRAU); Goereesoe farm next to R319 (–AC), 10 Aug 2017, *du Preez 313* (BOL); Goereesoe farm, Bredasdorp region (–AC), 26 Nov 2011, *Muasya, Stirton & Curtis 6303* (BOL); 10 Sept 2011, *Curtis 15* (BOL); Plaatjieskraal (–AC), 7 Dec 2011, *Muasya, Stirton & Curtis 6349* (BOL); Luipaardskop farm at N. foothills of Suikerkankop (–AD), 2 Oct 1983, *C.J. Burgers 3197* (NBG); Spitskop (east) farm near gum trees (–AD), 20 May 2017, *du Preez 281* (BOL); Haarwegskloof, north of research centre (–AD), 6 Aug 2017, *du Preez 308* (BOL); Bredasdorp, Haarwegskloof farm (–AD), 12 Oct 2011, *Stirton, Ibrahim & Curtis 13362* (BOL),; Haarwegskloof Nature Reserve (–AD), 8 Oct 2014, *Stirton 14059* (BOL); Nysty Farm, 40 km N of Bredasdorp, along San Souci road (–AD), 2 Dec 2011, *Stirton & Curtis 13442* (BOL); ; Farm Luipaardskop 53, about 12 km NW of

Wydgelee (–AD), 10 Sept 2000, *Helme 1760* (NBG), 2 Oct 1983, *C.J. Burgers 3197* (K, NBG); Farm Spitskop, near Ouplaas (–AD), 25 Sept 1986, *J. Albertyn 852* (NBG); 33 km NE of Bredasdorp on Plaatjieskraal farm (–AD), 1 Aug 2001, *Helme 2061* (NBG); Potberg NR, 1.5 km due west of Potberg Education Centre (–BC), 1 Sept 1983, *C.J. Burgers 3129* (K, NBG); 1 km west of Potberg centre, De Hoop (–BC), 10 Oct 1986, *B.-E. van Wyk 2128* (JRAU); Potberg NR, 50 m from western perimeter fence up gulley (–BC), 11 Jul 2018, *du Preez 516* (BOL).

11. Polhillia involucrata (Thunb.) B.-E.van Wyk & A.L. Schutte; Kew Bulletin 44: 3 (1989). *Argyrolobium involucratum* (Thunb.) Harv. Fl. Cap. 2: 75 (1862). *Genista involucrata* (Thunb.) Briq. Étude Cytises Alpes Mar. 119 (1894). *Melolobium involucratum* (Thunb.) C.H.Stirt. S. Afr. J. Bot. 52: 4 (1986). *Psoralea involucrata* Thunb. Prodr. 136 (1800); Fl. Cap. 607 (1823). Type: South Africa, Cape, without precise location, Thunberg s.n. (UPS-THUNB 17575!, lectotype, chosen by Stirton, 1986b).

Plant an erect, rounded shrub, 0.4–1.0 (–1.5) m tall, sprouting from woody rootstock. *Branches* greyish-brown, smooth, densely villous on young stems, sparsely villous on old stems, lenticels absent. *Stipules* 4.0–10.0 mm long, deltoid-oblong, bifurcate, fused only around base, glabrous on outside, villous inside, with 3 prominent veins. *Leaves* digitately trifoliolate, arcuate, glabrous, sparsely hairy on abaxial surface and margin of young leaves only; midrib sunken; petiole shorter than lamina, 1.0–3.0 mm long; leaflets obovate, conduplicate, partially-flattened during wet season, apex apiculate-acute, base broadly attenuate, margin entire; terminal leaflet 3.0–9.0 × (1.0–) 2.0–4.0 (–6.0) mm; lateral leaflets symmetrical, 2.5–8.0 × 1.0–4.0 (–5.5) mm. *Inflorescence* terminal cluster of 2–5 (–6) flowers, subtended by enlarged stipules that look like involucral bracts; pedicel up to 6.0 mm long, villous; bracts unknown, likely highly reduced and caducous; ebracteolate. *Flowers* pink or rarely white, 10.0–12.0 mm long. *Calyx* campanulate, ± 7.0 mm long, bilabiate, papery thin, flushed red, sparsely pilose, denser on and inside teeth; tube 4.0–5.0 mm long; dorsal lip bifid, teeth triangular, 2.0–3.0 mm long, fused for less than one third their length, tips acute; ventral lip trifid, teeth narrowly triangular-triangular, 2.0–3.0 mm long, fused for ± half their length, spreading away from petals, tips acute; inner tips of teeth pilose. *Standard petal* 11.0–12.0 mm long, claw lanceolate, about 4.5 mm long, lamina broadly elliptic, apex emarginate, base broadly attenuate, 6.5–7.5 × 6.0–7.0 mm, pilose abaxially along midrib.

Wing petals 10.0–11.0 mm long, equal to or slightly longer than keel; claw (3.0–) 4.0–4.5 mm long; lamina broadly oblong, apex rounded-obtuse, base truncate, 7.0–7.5 × 3.5–4.0 mm, glabrous, oval depression above with lamellate sculpturing dorsal basal-mid, becoming sparser towards the apex. *Keel petals* 9.0–11.0 mm long; claw 4.0–4.5 mm long; lamina lunate, 6.2–7.0 × 3.0–3.5 mm, apex rounded-square almost at 90° angle, base truncate, pilose along lower half of lamina, pocket present, basal along midline. *Stamens* 10; anthers dimorphic, alternating between four long, basifixed anthers, 1.1–1.2 mm long, and one carinal anther, 0.9–1.0 mm long, and five short, dorsifixed anthers, 0.6–0.7 mm long; filaments fused forming staminal tube, 8.9–9.1 mm long, slit dorsal, unfused portion of filaments remaining equidistant, sub-erect, loosely arranged around style. *Pistil* 10.1–11.5 mm long, subsessile; ovary narrowly oblong, 5.7–6.3 mm long, 0.9–1.1 mm wide, sericeous, ovules 7; style terete, sub-erect, usually at about 60–70° angle, height of curvature 1.8–2.2 mm, glabrous, except basally; stigma regular, capitate. *Pod* dehiscent, opening along two seams, oblong, non-plicate, laterally compressed between seeds, tapering toward the apex and base, woody, sericeous to somewhat glabrescent, pale beige, 23.0–29.0 × 3.0–4.5 mm. *Seed* ovate-squared; testa olive-green to dark-brown, marbled pale orange, smooth, 2.4–2.7 mm long, 2.1–2.4 mm wide, 1.5–1.9 mm thick; hilum round, recessed, sub-terminal; rim aril inconspicuous; microphyll hidden. *Pollen* tricolpate; oblong to ovate in equatorial view, rounded-triangular in polar view, polar apices rounded; roughly 25.0–33.0 × 17.0–21.0 µm in size; semi-tectate, tectum micro-reticulate to finely-reticulate, lumina heterobrochaete, rounded to oblong; pollen kit ample; intra-luminary bacules present

Flowering time: October to December

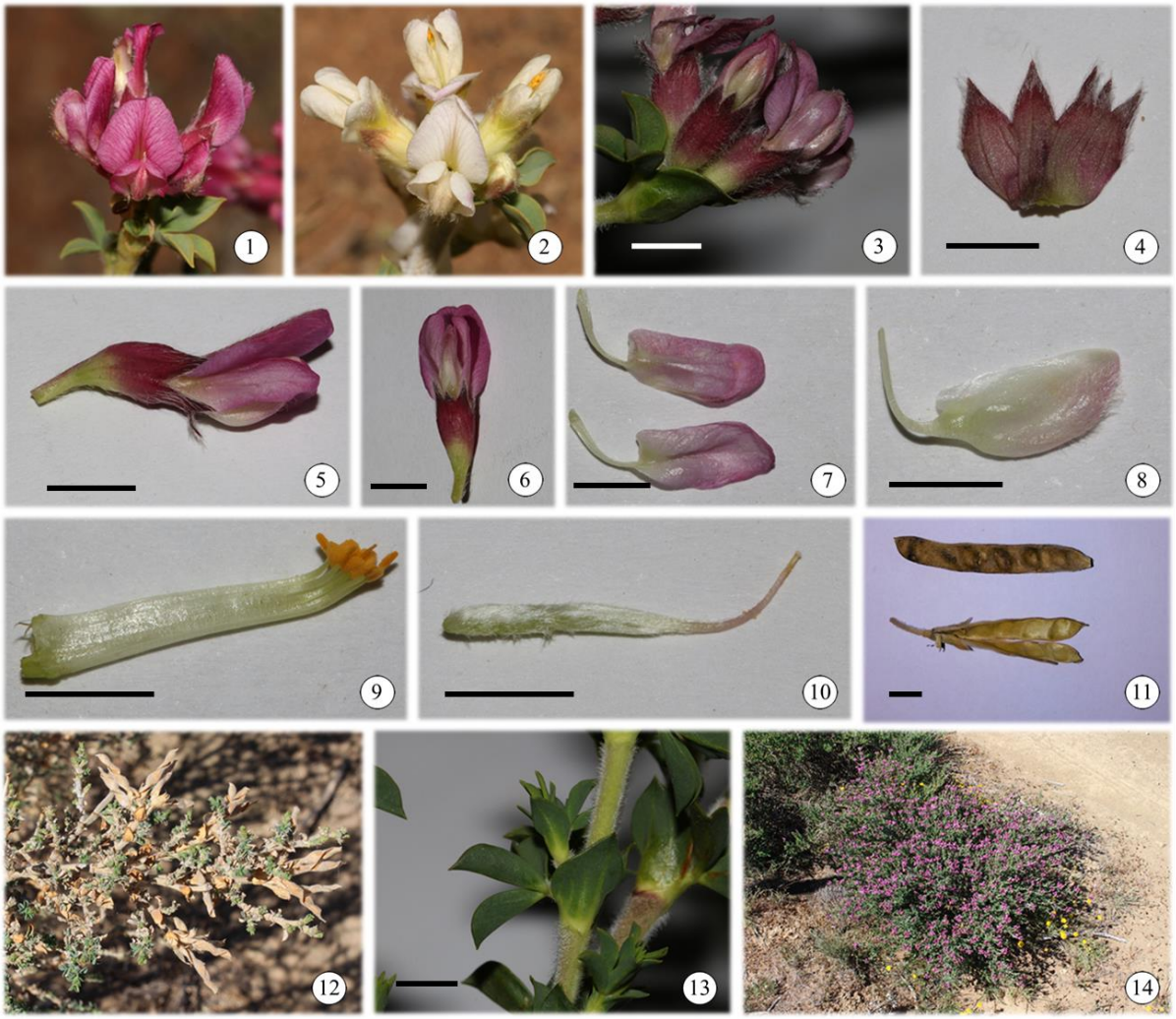

Figure 29: Composite plate of *Polhillia involucrata*. 1 – Inflorescence with pink flowers; 2 – Inflorescence with white flowers; 3 – Side view of inflorescence showing pink-flushed sepals; 4 – Calyx; 5 – Flower side view; 6 – Flower bottom view; 7 – Wing petals; 8 – Keel petal; 9 – Androecium; 10 – Gynoecium; 11 – Pods; 12 – Dehiscent pods on bush; 13 – Close-up of leaves and stipules; 14 – Habit. Scale bars: 4 mm. Photographs by B. du Preez.

Etymology

The specific epithet "involucrata" refers to the enlarged terminal stipules that surround flowers in the bud stage in a way similar to an involucral bract.

Diagnostic characters

Polhillia involucrata (Figure 29) is characterized by having pink flowers, which is unique among southern African Genisteae, of which flowers are primarily yellow, rarely white. The glabrous or rarely sparsely pilose leaves and stipules (*versus* pubescent leaves and stipules in other *Polhillia* species), enlarged terminal stipules (*versus* small terminal stipules), and

dehiscent fruit (*versus* indehiscent fruit) are also diagnostic of this species, and distinguishes it from all other *Polhillia* species.

Distribution, ecology and conservation status

This species was previously only thought to occur in two regions of the Roggeveld escarpment (Figure 30) where it grows in Roggeveld Shale Renosterveld (FRs 3) (Mucina and Rutherford, 2006). An isolated collection of this species was, however, made in 2003 (DGE818) along the R318 between Touwsriver and Montagu. The more widespread occurrence of the species in this area was confirmed when flowering specimens were discovered and collected in October 2017 (BDP397) along the road several kilometres north of the 2003 collection in Matjiesfontien Shale Renosterveld (FRs 6) (Mucina and Rutherford, 2006). This represents only the third known location of this species. The species may be more widespread and thus further exploration, especially after a fire, may yield more populations. It appears to favour relatively flat areas at high altitude (above 1000m) shale Renosterveld where it is relatively cool and moist. The generation length for this species is unknown, but is expected to be less than 50 years (Raimondo *et al*., 2009). It is threatened largely by overgrazing, habitat loss due to ploughing of new lands, and a lack of fire in most areas. Climate change is also likely to threaten the long-term survival of this species due to the lack of suitable habitat remaining around existing populations. The population at Kanariesfontein is on the brink of extinction, as there are only about 10 individuals left, that are annually grazed, the vegetation is > 60 years old and the area is drier that the other two areas. The population on the road verge around Blomfontein is large with several hundred plants that look healthy. It has burnt recently and has a low browsing pressure. The population of 6 plants were found along the R318 growing in a road cutting. The Red Data List status of this species is assessed as EN B1ab(i,ii,iii,iv,v)+B2ab(i,ii,iii,iv,v) following the latest IUCN categories and criteria (IUCN, 2012).

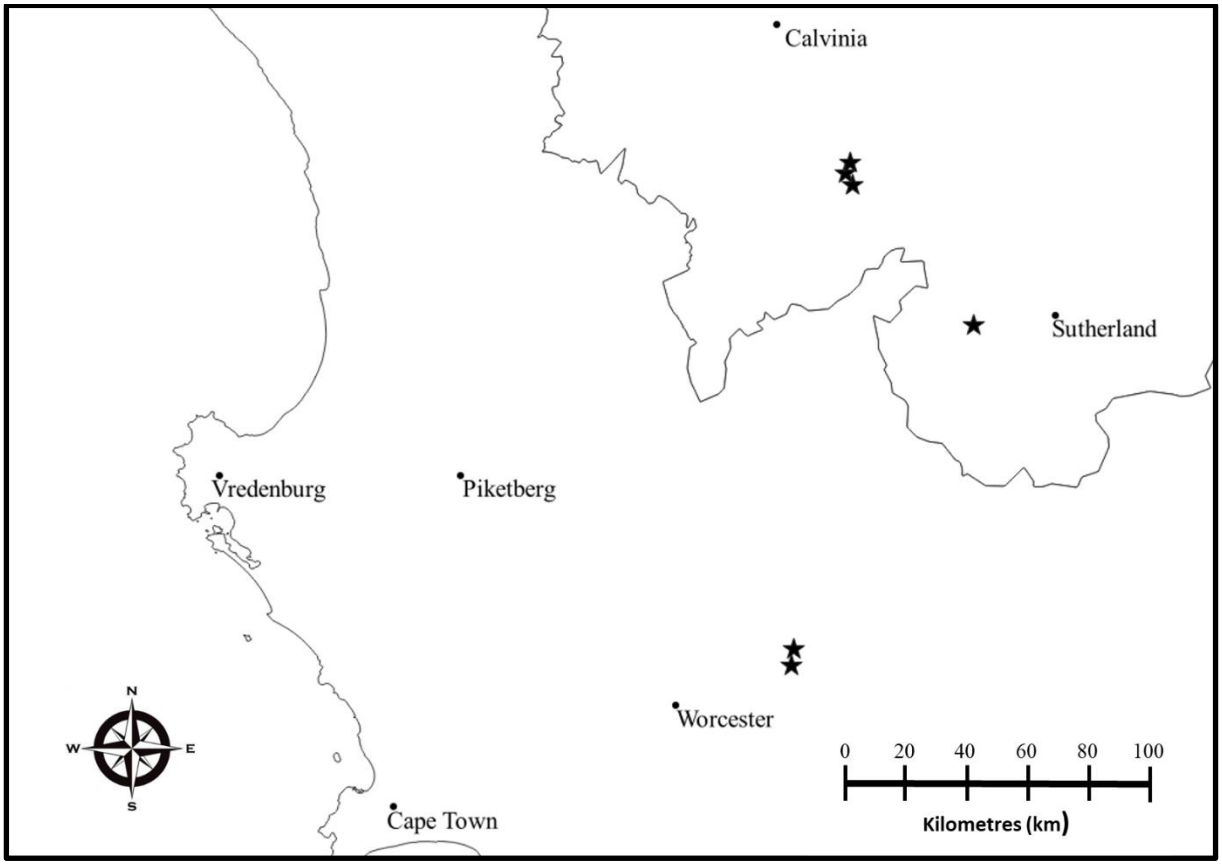

Figure 30: Distribution of *Polhillia involucrata*.

Additional specimens examined

South Africa. NORTHERN CAPE: **3119 (Calvinia):** Roggeveld, turnoff to Blomfontein, in road reserve (–DD), 26 Sept 2009, *Helme 6189* (NBG); Roggeveld, along Rooiwal road to Middelpos, Blomfontein (–DD), 27 Sept 2000, *H. Rosch 793* (NBG); Farm Blomfontein, Roggeveld escarpment (–DD), 7 Jan 1986, *C.H. Stirton 10999* (NBG); Entrance to Blomfontein farm (–DD), 27 Oct 1983, Snijman 778 (NBG); 23 Apr 2017, *du Preez 279* (BOL); 23 Oct 2017, *du Preez 405* (BOL), *406* (BOL); 17 Dec 1985, *A.L. Schutte 397* (JRAU), *398* (JRAU); Nov 1986, *M. Steenkamp s.n. sub A.L. Schutte 377* (JRAU), *379* (JRAU); **3120 (Williston):** 68km from Calvinia on Blomfontein road to Middelpos (–CC), 15 Dec 1976, *Goldblatt 4614* (NBG); Between Calvinia and Middelpos on Blomfontein road, about 70km SE Calvinia (–CC), 26 Oct 1976, *Goldblatt 4389* (BOL, NBG); Droëkloof farm along Blomfontein road (–CC), 23 Oct 2017, *du Preez 402* (BOL, K, NBG), 403 (BOL, NBG); 25km west of Middelpos, near Bo Tuin farm, Roggeveld Escarpment (–CC), 14 Nov 1987, Goldblatt & Manning 8652 (PRE); Kareebank farm south of Blomfontein on Roggeveld escarpment (–CC), 23 Oct 2017, *du Preez 407* (BOL); **3220 (Sutherland):**

Sutherland – Kanariesfontein (–AD), Oct 1990, *Bayer 6142* (PRE); Kanariesfontein farm behind *Populus* plantation, Sutherland (–AD), 16 Sept 2011, *Boatwright 602* (NGB); 24 Oct 2017, *du Preez 409* (BOL); **3319 (Touwsriver):** Road cutting along R318 before tower (–BD), 21 Oct 2017, *du Preez 397* (BOL); Next to R318 (–BD), 25 Mar 2003, *Gwynne-Evans 818* (BOL).

7. ACKNOWLEDGEMENTS

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Declaration by the candidate (for chapter prepared for Journal of Plant Biology)

With regards to Chapter 4 (**RARE AND LIVING ALONE - DO EDAPHIC FACTORS CAUSE ALLOPATRY OF *POLHILLIA* C.H.STIRT. SPECIES?**), the nature and scope of my contribution were as follows:

Nature of contribution	**Extent of contribution (%)**
Data collection, analysis, interpretation and manuscript preparation	80

The following co-authors have contributed to Chapter 4:

Name	**e-mail address**	**Nature of contribution**	**Extent of contribution (%)**
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Signature of candidate: .....…Brian du Preez…….

Declaration by co-authors:

The undersigned hereby confirm that

5. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 4.
6. No other authors contributed to Chapter 4 than those specified above.
7. There are no conflicts of interest relevant to Chapter 4 of this dissertation.

Signature	**Institutional affiliation**	**Date**
Léanne L. Dreyer	Stellenbosch University	December 2018
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CHAPTER 4

RARE AND LIVING ALONE - DO EDAPHIC FACTORS CAUSE ALLOPATRY OF POLHILLIA C.H.STIRT. SPECIES?

ABSTRACT

Polhillia C.H.Stirt. is a small genus of legumes that was recently revised. It comprises 11 species endemic to Renosterveld vegetation within the Greater Cape Floristic Region (GCFR). Most species occur in geographically small areas, with six species restricted to the Overberg region of the Western Cape Province. Interestingly, none of these species ever occur in sympatry, even where species distributions overlap. Little is known about the edaphic conditions in which *Polhillia* species grow, but field observations highlighted that edaphic heterogeneity may influence the distributions of species, especially within the Overberg. Through observing different habitat parameters, soil analyses and identifying rhizobial symbionts of eight of the eleven *Polhillia* species, we gained novel insights into the ecology of these previously poorly studied species. We found that environmental heterogeneity in the form of different landscape topological features and differences in soil parameters are important factors that inhibit sympatry where distributions overlap. We speculate that such differences may have led to the divergence of species even in cases where distances are short enough to allow dispersal. Although rhizobial symbionts do not appear to play a role in influencing *Polhillia* species distributions, it was interesting that we found *Polhillia* to be nodulating with *Rhizobium* and three distinct lineages of *Mesorhizobium* represented. This contrasts to bacteria in the nodules of *Argyrolobium*, the only Cape Genisteae genus studied so far, from which only *Mesorhizobium* (*α*-proteobacteria) have been reported from within the GCFR.

1. INTRODUCTION

Polhillia C.H.Stirt. is a small genus of papilionoid legumes endemic to the Greater Cape Floristic Region (GCFR) of South Africa (Manning and Goldblatt, 2012; Snijman, 2013). Eleven species are now recognised in the genus (du Preez *et al*. 2019 – Chapter 3). This genus has until recently been poorly known, not only in terms of taxonomy, but also in terms of ecology and factors that determine the distribution of species. All species occur in Renosterveld vegetation, a very diverse, but also threatened, habitat (Cowling *et al.*, 1986; Cowling and Holmes, 1992; Kemper *et al.*, 1999; Raimondo *et al.*, 2009; Curtis *et al.*, 2013). The nutrient rich soils that typically are home to this broad vegetation type have been extensively transformed into crop fields (Cowling *et al.*, 1986; Kemper *et al.*, 1999). As such, all *Polhillia* species are threatened with extinction and the genus is recognised as the third most threatened plant genus in South Africa (Raimondo *et al.*, 2009; Ebrahim *et al.*, 2016; Magee *et al.*, 2017). The Overberg region, known for its extensive wheat and canola fields, is also the centre of diversity of the genus (du Preez *et al*. 2019 – Chapter 3; Kemper *et al.*, 1999). Six of the 11 recognised species occur within a relatively small area, but species never occur in sympatry (du Preez *et al*. 2019 – Chapter 3).

The GCFR is rich in both plant and geological diversity, with many species being narrow endemics to specific edaphic conditions (Cowling and Holmes, 1992; Linder, 2003; Manning and Goldblatt, 2012; Snijman, 2013). Edaphic heterogeneity has also been proposed as a mode of speciation for plants and animals around the world, including the GCFR (Linder and Vlok, 1991; Cowling and Holmes, 1992; Wiens and Graham, 2005; Kozak and Wiens, 2006). The GCFR is dominated by two biomes; Fynbos typically growing on nutrient poor, acidic soils derived from sandstone, and Succulent Karoo growing on fertile, shale derived soils (Rebelo *et al.*, 2006; Manning and Goldblatt, 2012; Snijman, 2013). Renosterveld can be considered a transitional vegetation between Fynbos and Succulent Karoo vegetation, and grows on fairly fertile duplex soils derived from shale deposits (Thwaites and Cowling, 1988; Cowling and Holmes, 1992; Kemper *et al.*, 1999). Renosterveld typically occurs in lowland habitats (Levyns, 1956; Rebelo, 1996), but can also occur on relatively high plateaus such as the Roggeveld escarpment (Clark *et al.*, 2011; Van der Merwe and van Rooyen, 2011; Snijman, 2013). Soils in these regions are typically not well studied, but are known to have higher concentrations of Phosphorus (P) compared to soils associated with Fynbos vegetation

(Witkowski and Mitchell, 1987). Soil type and nutrient characters vary in the landscape and often lead to the localization of species (Cowling, 1990; Cowling and Holmes, 1992). The type of soil, soil pH and the concentrations of macro-nutrients, especially Nitrogen (N) and P, greatly influence the distribution of plant species and vegetation community boundaries (Witkowski and Mitchell, 1987; Thwaites and Cowling, 1988; Richards *et al.*, 1997). Soil pH also affect plant species composition and plant growth across the landscape (Amacher *et al.*, 2007; Wagner *et al.*, 2013). Renosterveld soils have higher pH levels (5.9–6.9) (Solomon, 2015) than acidic Fynbos soils (pH of 3.9–4.3) (Mitchell *et al.*, 1984). Other important minerals that can vary considerably in the landscape include Calcium (Ca), Magnesium (Mg), Sodium (Na) and Potassium (K) (Richards *et al.*, 1997). On the Cape Peninsula it was found that legume distributions were attributed to different concentrations of clay, K, S, Fe and Zn (Dludlu *et al.*, 2018a). Nothing though is known about how soils and soil elements influence the distribution of *Polhillia* species within the landscape and whether some or all species have specific soil preferences.

Legumes are renowned for their ability to fix atmospheric nitrogen into the soil for plant use (Gualtieri and Bisseling, 2000; Sprent, 2007; Sprent *et al.*, 2013; Walker *et al.*, 2015). Most legumes in the subfamilies Papilionoideae and Mimosoideae form symbiotic relationships with gram-negative bacteria called rhizobia on the root surface where nodules form as sites of N-fixation (Gualtieri and Bisseling, 2000; Sprent, 2007; Sprent *et al.*, 2013). N-fixation is less common in the subfamily Caesalpinioideae (Sprent, 2007; Sprent *et al.*, 2013; Walker *et al.*, 2015). Rhizobia are classified into two groups; *α*-proteobacteria and *β*-proteobacteria (Gupta, 2000; Sawada *et al.*, 2003; Masson-Boivin *et al.*, 2009; Walker *et al.*, 2015). Knowledge of rhizobial diversity has increased considerably over the past few decades. The *α*-proteobacteria first only included the genus *Rhizobium*, but it has now been split into multiple genera (Willems, 2006; Masson-Boivin *et al.*, 2009). The recent discovery of widespread and diverse *β*-proteobacteria such as *Burkholderia* has further added to knowledge of legume-bacteria symbiosis in biological N fixation (Willems, 2006; Masson-Boivin *et al.*, 2009; Gyaneshwar *et al.*, 2011; Lemaire *et al.*, 2015; Walker *et al.*, 2015). Rhizobial diversity has been extensively studied in a variety of Cape legumes (*e.g.* Dludlu *et al.*, 2018b; Dludlu *et al.*, 2018c; Garau *et al.*, 2009; Howieson *et al.*, 2013; Kanu and Dakora, 2012; Kock, 2004; Lemaire *et al.*, 2015; Steenkamp *et al.*, 2015). Cape legumes house a large diversity of rhizobia, with distinct patterns of rhizobial preference between different legume groups (Lemaire *et al.*, 2015; Dludlu *et al.*, 2018b). This is especially prevalent in nutrient-

poor soils such as in the Fynbos, where in their study, Lemaire *et al.* (2015) found all studied legume species to nodulate. The acidic and oligotrophic nature of Fynbos soils in the GCFR have meant that legumes had to nodulate in order to provide usable soil nitrogen (Power *et al.*, 2010). Rhizobia sampling of GCFR Genisteae was under-represented in the Lemaire *et al.* (2015) study, which included only three *Argyrolobium* Eckl. & Zeyh. species and no *Polhillia*, *Melolobium* Eckl. & Zeyh. or *Dichilus* DC. taxa. From the limited data available on rhizobia nodulating Genisteae species, it appears that *α*-proteobacteria are favoured (Lemaire *et al.*, 2015). *Mesorhizobium* (Jarvis *et al.*, 1997) and *Bradyrhizobium* (Jordan, 1982) have been found to be associated with *Argyrolobium* in the GCFR and the SA Great Escarpment, respectively (Lemaire *et al.*, 2015; Beukes *et al.*, 2016). *Bradyrhizobium* has also been recorded nodulating with Genisteae in the Mediterranean region (Vinuesa *et al.*, 2005; Bourebaba *et al.*, 2016). It is thus important to investigate rhizobial symbiont diversity among *Polhillia* species, not only to fill part of the knowledge gap that exists among SA Genisteae and Renosterveld legumes in general, but also as rhizobial preference may help explain the distribution of *Polhillia* species.

With the revision of *Polhillia* in place (du Preez *et al*. 2019 – Chapter 3), potential underlying ecological and edaphic factors that influence the distribution and abundance of the different species can now be explored more accurately. We thus aim to investigate potential factors that may explain distribution patterns displayed by the different *Polhillia* species. We hypothesise that species occupy different specific niches and different soils and that one or both of these factors may be maintaining the allopatric distributions of species. We further hypothesise that rhizobial symbionts will comprise primarily *α*-proteobacteria, more specifically *Mesorhizobium*.

2. MATERIALS AND METHODS

2.1. Habitat assessments

Habitat assessments were done when visiting populations of all the different *Polhillia* species, with the following variables measured in all populations of all species: geology, topography, slope aspect, slope gradient and altitude in metres above sea level (m.a.s.l.).

2.2. Soil Analysis

2.2.1. Sampling and analysis

Soil samples were collected during extensive fieldwork for all 11 species recognized by du Preez *et al*. (2019 – Chapter 3). Collection details and abiotic attributes at each locality for the soil samples analysed are summarized in Table 1. A total of three soil samples were collected per location for up to three locations per species. Three samples per species were sent to the Elsenburg Plant, Water and Soil Science laboratory for analysis of soil pH, resistance, sand: silt: clay fractions, macro-mineral and NH_4 nitrogen concentrations. Three samples per species were also prepared and analysed at the University of Cape Town for percentage composition of minerals using an SPECTRO XEPOS X-ray Florescence (XRF) Spectrophotometer. XRF data were corrected using standardised correction values for all minerals analysed.

Table 1: Details of soil samples of the 11 *Polhillia* species used in our analyses, including GPS co-ordinates and altitude (m.a.s.l.), geology, slope aspect and gradient at collection sites.

Species	Sample number	Latitude	Longitude	Altitude (m a.s.l.)	Geology	Slope Aspect	Slope Gradient
P. brevicalyx	Brevi.1	-34.20007	20.24826	208	Shale	South	Gentle
	Brevi.2	-34.20345	20.25155	211	Shale	West	Moderate
	Brevi.3	-34.18938	20.26220	192	Shale	N/A	Flat
P. connata	Conn.1	-34.37025	20.27167	86	Shale	North	Moderate
	Conn.2	-34.35550	20.17394	117	Shale	West	Moderate
	Conn.3	-34.40059	20.15084	180	Shale	East	Moderate
P. groenewaldii	Groen.1	-33.98403	20.04713	169	Shale	South	Gentle
	Groen.2	-33.98403	20.04713	169	Shale	South	Gentle
	Groen.3	-33.98403	20.04713	169	Shale	South	Gentle
P. curtisiae	Curt.1	-34.27380	20.31578	194	Silcrete	North	Gentle
	Curt.2	-34.33060	20.32302	245	Shale	North	Gentle
	Curt.3	-34.31364	20.39460	275	Silcrete	West	Moderate
P. fortunata	Fort.1	-33.91600	21.43926	341	Shale	South	Moderate
	Fort.2	-33.91600	21.43926	341	Shale	South	Gentle
	Fort.3	-33.91600	21.43926	341	Shale	South	Gentle

P. ignota	Igno.1	-32.87173	18.79351	142	Alluvial	West	Gentle
	Igno.2	-32.69959	18.89731	111	Alluvial	South	Moderate
	Igno.3	-32.69959	18.89731	111	Alluvial	South	Moderate
P. involucrata	Inv.1	-31.94412	19.99150	1405	Shale	N/A	Flat
	Inv.2	-31.91343	20.00677	1409	Shale	N/A	Flat
	Inv.3	-32.42833	20.40027	1337	Shale	N/A	Flat
P. obsoleta	Obso.1	-33.66352	19.41816	207	Alluvial	N/A	Flat
	Obso.2	-33.66774	19.41192	203	Alluvial	N/A	Flat
	Obso.3	-33.66317	19.42244	207	Alluvial	N/A	Flat
P. pallens	Pall.1	-34.40777	20.81912	30	Shale	West	Moderate
	Pall.2	-34.28902	20.51062	118	Shale	West	Steep
	Pall.3	-34.26177	20.60726	73	Shale	South	Steep
P. stirtoniana	Stirt.1	-34.32101	20.27810	151	Shale	South	Moderate
	Stirt.2	-34.31246	20.39263	210	Shale	North	Moderate
	Stirt.3	-34.34637	20.35444	180	Shale	South	Steep
P. xairuensis	Xair.1	-34.08326	20.65077	233	Shale	West	Gentle
	Xair.2	-34.13486	20.72650	232	Silcrete	South	Moderate
	Xair.3	-34.18076	20.72449	208	Silcrete	South	Gentle

2.2.2. Statistical analysis

XRF and Elsenburg data were combined into a single dataset and some trace elements were removed. A Principal Component Analysis (PCA) was run in R Studio and plotted using the ggbiplot package to determine which elements influence the distribution of the various *Polhillia* species (R Development Core Team, 2015). One-way ANOVA's were conducted in Statistica for each soil component to compare concentrations of individual elements between all *Polhillia* species at the various localities (StatSoft. Inc., 2018). Preceding each ANOVA, a test for normality of data was done. Kruskal-Wallis ANOVA's were done to produce LS Means graphs with 95% confidence intervals. Levene's Test for homogeneity of variances was conducted to test the assumption that variances were equal. Least significant difference (LSD) *post hoc* tests were done to display species level comparisons for each soil parameter and element.

2.3. Rhizobia

Nodules could not be exhaustively collected in the field, as all species are resprouters, which made the collection of nodules near impossible. We thus cultivated *Polhillia* species under greenhouse conditions to harvest nodules for rhizobial identification. Soil and seeds were collected for 9 of the 11 species during 2017, as far as possible from type localities. Soil was placed in plastic growth plant bags in the greenhouse of the Department of Botany and Zoology, Stellenbosch University. Seed germination was activated by placing seeds in

boiling water and leaving them to soak overnight. Fifty seeds per species were then planted in their native soil in early spring for optimal growth. Nodules were harvested five months later and prepared for bacterial extraction. Nodules were first photographed and measured using a Leica M125 stereomicroscope with an attached Leica MC170 periscope camera, using LAS v4.9 software (Leica Microsystems, 2018). Nodules were then externally sterilized by dipping them in pure JIK® (a bleaching solution containing 3.5% (w/v) sodium hypochlorite) for 30–60 seconds, based on the size of nodules, followed by three successive rinses in distilled water (Vincent, 1970). Nodules were then crushed in 500 µl sterilized water and pipetted onto prepared Yeast Extract Mannitol Agar (YEMA) plates and incubated at 27°C for 7 days in a dark incubator. Cultures were sub-cultured onto fresh YEMA plates at least three times in order to obtain pure cultures. Total DNA extraction of all cultures was done using the CTAB method of Doyle and Doyle (1987). Primers 27F and 1492R were used to target the 16S rDNA region and PCR conditions were set at 95°C for 5 min initial denaturation, 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, followed by 72°C for 5 min final extension (Lane, 1991; Weisburg *et al.*, 1991). Primers RecA63F and RecA504R were used to target the recA region and PCR conditions were set at 95°C for 5 min initial denaturation, 30 cycles of 94°C for 45 s, 62°C for 1 min and 72°C for 90 s, followed by 72°C for 5 min final extension (Gaunt *et al.*, 2001). Sequencing reactions of PCR product were done at the Stellenbosch University Central Analytical Facility (CAF). Sequences were edited using Chromas2 v2.3 (Technelysium Pty Ltd, 2004), while sequences were aligned using BioEdit v7.2.5 (Ibis Biosciences, 2013). They were then blasted to GenBank to identify the rhizobial strains, after which the alignment was supplemented with other sequences from GenBank (Tables 2 and 3). Molecular phylogenetic analyses were done with Bayesian Inference (BI) using MrBayes v3.2 (Ronquist and Huelsenbeck, 2003) under nst=mixed model of nucleotide substitution and an invgamma parameter to account for among site rate variation, for 10^6 generations and trees were sampled every 2000 generations under otherwise standard settings. All runs reached stationarity on the posterior as judged by MrBayes internal diagnostics. Phylogenetic consensus trees were viewed and analysed using FigTree v1.4.3 (Rambaut, 2009).

Table 2: Additional GenBank sequences used to reconstruct the 16S rRNA phylogeny of *Polhillia* rhizobial symbionts.

Sequence Name	**GenBank Accession**	**Reference**
Bradyrhizobium sp. MM5405	KF802609.1	Lemaire *et al*., 2015
Rhizobium sp. OD123	KF802610.1	Lemaire *et al*., 2015
Rhizobium sp. OD49	KF802576.1	Lemaire *et al*., 2015
Rhizobium sp. strain N9	MF944252.1	Lunga *et al.* unpublished
Rhizobium leguminosarum strain S11190	MF977616.1	Jorrin and Imperial, unpublished
Rhizobium sp. tg2	KX417630.1	Lasa *et al*., unpublished
Rhizobium sp. strain NAK 312	KY921866.1	Mwenda *et al*., unpublished
Mesorhizobium sp. strain WSM3267	MF949017.1	De Meyer *et al*., unpublished
Mesorhizobium sp. MM5361	KF802569.1	Lemaire *et al*., 2015
Mesorhizobium sp. MM5757	KF802575.1	Lemaire *et al*., 2015
Mesorhizobium sp. MM6354	KF802607.1	Lemaire *et al*., 2015
Mesorhizobium sp. OD52	KF802606.1	Lemaire *et al*., 2015
Mesorhizobium sp. MM5333	KF802594.1	Lemaire *et al*., 2015

Table 3: Additional GenBank sequences used to reconstruct the recA phylogeny of *Polhillia* rhizobial symbionts.

Sequence Name	**GenBank Accession**	**Reference**
Bradyrhizobium sp. MM5621	KF802770.1	Lemaire *et al.*, 2015
Rhizobium sp. RPVR04	GQ863532.1	García-Fraile *et al.*, 2010
Rhizobium leguminosarum bv. viciae strain UPM791 RecA	KY307856.1	Rubio-Sanz *et al.* unpublished
Rhizobium leguminosarum RVS03	FJ596031.1	Álvarez-Martínez *et al.*, 2009
Rhizobium sp. strain NAK288	MG288726.1	Mwenda *et al.* unpublished
Rhizobium sp. strain NAK315	MG288729.1	Mwenda *et al.* unpublished
Rhizobium sp. 952N3	KT719108.1	Lemaire *et al.* unpublished
Rhizobium sullae strain_IS123	FJ816279.1	Han *et al.* unpublished
Rhizobium sp. OD49	KF802763.1	Lemaire *et al.*, 2015
Mesorhizobium loti strain LMG6125	AM076372.1	Barlow *et al.* unpublished
Mesorhizobium erdmanii USDA3471	AJ294371.1	Gaunt *et al.*, 2001
Mesorhizobium sp. MM5364	KF802793.1	Lemaire *et al.*, 2015
Mesorhizobium sp. MM5352	KF802760.1	Lemaire *et al.*, 2015
Mesorhizobium sp. CCANP1	HG323883.1	Armas-Capote *et al.*, 2014
Mesorhizobium sp. OD108	KF802755.1	Lemaire *et al.*, 2015
Mesorhizobium sp. MM5333	KF802780.1	Lemaire *et al.*, 2015
Mesorhizobium australicum LMG24608T	FR863559.1	De Meyer *et al.*, 2011

3. RESULTS

3.1. Habitat

The known distribution of the 11 *Polhillia* species currently recognized is summarised in Figure 1. The genus *Polhillia* is endemic to the Greater Cape Floristic Region *sensu* Manning and Goldblatt (2012), with all 11 species occurring within the Core Cape Subregion (CCR) and one extending into the Extra Cape Subregion (ECR) *sensu* Snijman (2013). Figure 1 shows that *Polhillia brevicalyx* (C.H.Stirt.) B.-E.van Wyk & A.L.Schutte, *Polhillia connata* (Harv.) C.H.Stirt., *Polhillia curtisiae* C.H.Stirt. & Muasya, *Polhillia pallens* C.H.Stirt., *Polhillia stirtoniana* B.du Preez and *Polhillia xairuensis* B.du Preez are restricted to the Overberg Region of the Western Cape Province; most of them further restricted to very localized areas within the Overberg. *Polhillia groenewaldii* B.du Preez and *Polhillia fortunata* B.du Preez are new species (du Preez *et al*. 2019 – Chapter 3) that are highly localized and each known from single localities just outside of the Overberg. In contrast, *Polhillia ignota* Boatwr. and *Polhillia obsoleta* (Harv.) B.-E.van Wyk occur northeast of the Overberg in the Swartland and Worcester areas, respectively. *Polhillia ignota*, once considered to be extinct (Boatwright, 2010), is rather widely distributed in the Swartland, while *P. obsoleta* is highly localized around Worcester. *Polhillia involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte is the only species to occur in both the CCR and ECR, with the majority of populations occurring on the Roggeveld Escarpment. This species has the widest distribution, with southern populations separated from populations on the Roggeveld Escarpment by more than 100 km.

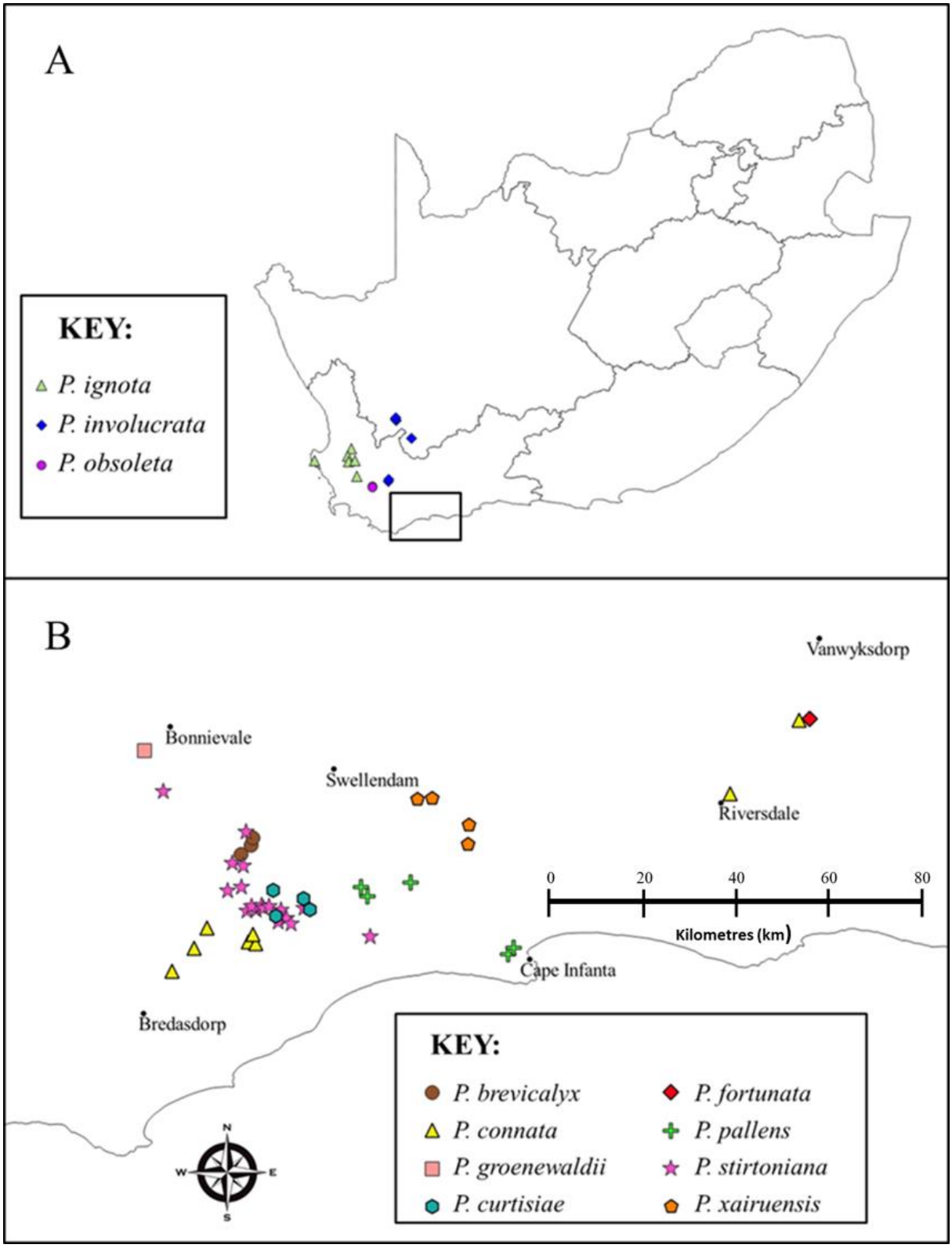

Figure 1: Map showing the distribution of all 11 *Polhillia* species (A) with emphasis on the dense clustering of species in and around the Overberg region (B). Note that most species are highly localized and species distributions seldom overlap.

All *Polhillia* species grow in Renosterveld vegetation, but the exact geology and position of species in the landscape differs (Table 4). This was especially true for Overberg species with narrow and often overlapping distributions.

Table 4: Summary of habitat observation data collected for all *Polhillia* species.

Species	Geology	Topography	Aspect	Slope	Altitude (m.a.s.l.)
P. brevicalyx	Shale	Slope	Variable	Gentle	±200
P. connata	Shale	Slope / gulley	Variable	Moderate–Steep	50–180
P. curtisiae	Shale / Silcrete	Slope	North	Gentle	190–275
P. fortunata	Shale	Slope / gulley	South	Moderate	±340
P. groenewaldii	Shale	Slope	South	Gentle	±170
P. ignota	Alluvial	Slope / flats	Variable	Flat–Moderate	50–120
P. involucrata	Shale	Slope / flats	Variable	Flat–Gentle	1000–1400
P. obsoleta	Alluvial	Flats	N/A	Flat	±200
P. pallens	Shale	Slope / gulley	South / West	Moderate–Steep	1–150
P. stirtoniana	Shale	Slope / gulley	Variable	Moderate-Steep	100–300
P. xairuensis	Shale / Silcrete	Slope / gulley	South / West	Moderate	200–280

Overall the habitat requirements of the different *Polhillia* species are rather similar, but for species with overlapping distributions such as *P. curtisiae* and *P. stirtoniana* as well as *P. brevicalyx* and *P. stirtoniana*, there are clear differences in habitat preferences. *Polhillia curtisiae* grows on shales or silcrete on gentle north facing slopes at an altitude of 190–275 m.a.s.l., while *P. stirtoniana* is restricted to shales on moderate south facing slopes or gullies at altitudes of 100–300 m.a.s.l. *Polhillia brevicalyx* is restricted to a single river course, while *P. stirtoniana* is found in gullies and steeper slope in the hills. The distribution of *P. fortunata* and *P. connata* appear to overlap in the Karoo, but as the *P. connata* population in this area has yet to be rediscovered, we cannot compare habitat preference between these two species. Species such as *P. pallens* and *P. stirtoniana* that do not show overlapping distributions obviously do not compete for habitat, which may explain their similar habitat requirements. It is interesting to note that *P. involucrata* is the only high-altitude species,

while all others occur in lowland habitats. Species growing in gullies were also found to include more individuals per population and generally had more sub-populations. Species growing on flats and gentle slopes appeared to have been more susceptible to ploughing, are generally rarer, and occur in fewer sub-populations.

3.2. Soil Analysis

Soils from various sites of all *Polhillia* species were analysed for numerous parameters including pH, Resistance, Sand; Silt; Clay fractions, NH4, total cations and concentrations of macro- and micro- elements. The results of the soil analyses are presented in Tables 5.1–5.4 and are used for both the one-way Anova's and PCA in this section.

Table 5.1: Summary of Elsenburg and XRF soil data for all 11 *Polhillia* species. Parameters investigated include: Soil pH, Resistance, Sand: Silt: Clay fraction, Ammonium (NH_4), Total cations, Sodium (Na), Magnesium (Mg) and Aluminium (Al). Brevi. = *Polhillia brevicalyx*; Conn. = *Polhillia connata*; Curt. = *Polhillia curtisiae*; Pall. = *Polhillia pallens*; Stirt. = *Polhillia stirtoniana*; Xair. = *Polhillia xairuensis*; Fort. = *Polhillia fortunata;* Groen. = *Polhillia groenewaldii*; Igno. = *Polhillia ignota*; Inv. = *Polhillia involucrata*; Obso. = *Polhillia obsoleta*.

Sample	pH (KCl)	Resistance (Ohm)	Sand %	Silt %	Clay %	NH_4 %	T-value (cmol/kg)	Na (mg/kg)	Mg (cmol/kg)	Al %
Overberg										
Brevi.1	6.9	510	75	14	11	0.346	16.0	154	0.025	8.1
Brevi.2	5.2	460	83	8	9	0.503	13.5	79	0.025	7.8
Brevi.3	5.2	530	65	18	17	0.474	14.9	151	0.002	7.9
Conn.1	5.4	1780	71	18	11	0.156	7.4	56	0.146	9.6
Conn.2	5.6	420	71	18	11	0.336	13.0	176	0.270	8.8
Conn.3	6.2	340	81	10	9	0.290	13.4	325	0.002	8.9
Curt.1	5.2	190	81	10	9	0.327	14.6	169	0.040	9.3
Curt.2	6.3	120	75	14	11	0.526	25.8	585	0.002	11.5
Curt.3	6.1	380	83	8	9	0.490	19.3	195	0.002	8.4
Pall.1	6.9	400	73	18	9	0.529	23.1	139	0.568	9.5
Pall.2	6.6	370	63	22	15	0.508	19.7	264	0.518	10.0
Pall.3	5.8	310	67	18	15	0.451	15.3	177	0.013	7.5
Stirt.1	5	320	75	14	11	0.314	13.1	211	0.002	6.1
Stirt.2	5.1	420	81	10	9	0.497	16.3	229	0.002	6.1
Stirt.3	6	460	65	20	15	0.610	19.7	117	0.525	9.9
Xair.1	5.5	460	79	12	9	0.639	19.9	312	0.002	6.7
Xair.2	5.4	1700	67	18	15	0.420	13.4	119	0.002	7.6
Xair.3	5.1	1070	57	26	17	0.284	9.8	107	0.350	7.3
Outside Overberg										
Fort.1	7	660	41	40	19	0.348	18.5	282	0.501	9.9
Fort.2	7.4	50	41	40	19	0.389	26.9	865	0.386	9.7
Fort.3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.357	9.2
Groen.1	7.9	280	75	14	11	0.371	22.7	233	0.662	9.9
Groen.2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.542	9.6
Groen.3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.551	9.8
Igno.1	5.3	240	87	6	7	0.815	19.1	157	0.002	6.1
Igno.2	4.9	520	89	4	7	0.245	8.4	30	0.002	5.3
Igno.3	5.3	440	89	4	7	0.205	8.3	53	0.002	6.5
Inv.1	7.1	830	81	10	9	0.112	10.9	47	0.411	8.5
Inv.2	5.8	1990	83	8	9	0.039	3.9	45	0.131	7.3
Inv.3	4.7	1430	83	8	9	0.086	3.7	12	0.140	7.0
Obso.1	5.3	290	87	6	7	0.362	11.7	83	0.278	7.3
Obso.2	5.5	280	71	12	17	0.210	7.8	88	0.297	8.5
Obso.3	4.8	400	73	12	15	0.173	6.9	74	0.272	7.5

Table 5.2: Summary of Elsenburg and XRF soil data of all 11 *Polhillia* species. Parameters investigated include: Silicon (Si), Phosphorus (P), Sulphur (S), Potassium (K), Calcium (Ca), Titanium (Ti), Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe) and Cobalt (Co). Brevi. = *Polhillia brevicalyx*; Conn. = *Polhillia connata*; Curt. = *Polhillia curtisiae*; Pall. = *Polhillia pallens*; Stirt. = *Polhillia stirtoniana*; Xair. = *Polhillia xairuensis*; Fort. = *Polhillia fortunata;* Groen. = *Polhillia groenewaldii*; Igno. = *Polhillia ignota*; Inv. = *Polhillia involucrata*; Obso. = *Polhillia obsoleta.*

Sample	Si %	P %	S %	K %	Ca %	Ti %	V %	Cr %	Mn %	Fe %	Co %
Overberg											
Brevi.1	24.4	0.042	0.036	1.841	0.365	0.489	0.0040	0.061	0.033	2.831	0.0016
Brevi.2	21.8	0.046	0.057	1.867	0.307	0.450	0.0049	0.056	0.023	2.951	0.0016
Brevi.3	26.8	0.034	0.032	1.857	0.226	0.493	0.0057	0.067	0.027	2.168	0.0007
Conn.1	26.3	0.038	0.020	2.111	0.182	0.562	0.0035	0.002	0.009	3.931	0.0018
Conn.2	24.2	0.040	0.040	2.372	0.322	0.515	0.0049	0.001	0.016	3.451	0.0005
Conn.3	26.6	0.043	0.049	1.471	0.329	0.526	0.0036	0.003	0.002	1.843	0.0002
Curt.1	24.7	0.046	0.085	2.089	0.418	0.547	0.0052	0.003	0.004	3.796	0.0020
Curt.2	24.2	0.035	0.059	2.290	0.445	0.577	0.0087	0.005	0.006	1.487	0.0005
Curt.3	23.7	0.047	0.078	1.299	0.669	0.541	0.0051	0.003	0.003	4.234	0.0002
Pall.1	19.9	0.031	0.056	2.480	0.751	0.494	0.0058	0.055	0.019	3.732	0.0017
Pall.2	21.5	0.025	0.031	2.907	0.459	0.515	0.0078	0.046	0.016	4.353	0.0014
Pall.3	21.6	0.026	0.030	1.711	0.306	0.312	0.0052	1.358	0.093	4.832	0.0049
Stirt.1	19.2	0.026	0.039	0.816	0.170	0.276	0.0049	1.339	0.092	3.643	0.0038
Stirt.2	16.7	0.029	0.034	0.882	0.179	0.278	0.0036	1.160	0.079	4.778	0.0039
Stirt.3	24.8	0.035	0.037	2.798	0.399	0.557	0.0080	0.004	0.013	4.191	0.0005
Xair.1	25.0	0.038	0.050	1.259	0.251	0.462	0.0058	0.055	0.025	2.190	0.0015
Xair.2	29.8	0.033	0.030	1.693	0.215	0.510	0.0038	0.077	0.017	1.865	0.0005
Xair.3	25.6	0.032	0.017	1.781	0.162	0.421	0.0038	0.049	0.035	3.212	0.0016
Outside Overberg											
Fort.1	26.1	0.067	0.015	2.799	0.334	0.585	0.0073	0.004	0.030	5.720	0.0005
Fort.2	26.9	0.053	0.072	2.848	0.314	0.589	0.0072	0.003	0.082	5.584	0.0011
Fort.3	26.9	0.038	0.008	2.497	0.236	0.608	0.0064	0.004	0.013	4.139	0.0007
Groen.1	25.7	0.042	0.018	2.641	0.342	0.561	0.0046	0.004	0.030	3.948	0.0005
Groen.2	24.5	0.042	0.016	2.570	0.192	0.553	0.0050	0.002	0.024	3.904	0.0005
Groen.3	24.9	0.039	0.016	2.626	0.190	0.574	0.0044	0.003	0.025	4.025	0.0009
Igno.1	21.4	0.068	0.173	1.053	0.889	0.344	0.0019	0.141	0.021	1.652	0.0002
Igno.2	29.2	0.035	0.065	1.039	0.274	0.482	0.0018	0.134	0.017	1.165	0.0002
Igno.3	29.8	0.038	0.055	1.054	0.336	0.435	0.0008	0.002	0.012	0.783	0.0002
Inv.1	26.1	0.085	0.019	2.321	0.563	0.402	0.0032	0.002	0.039	2.139	0.0002
Inv.2	30.9	0.045	0.000	2.017	0.280	0.363	0.0018	0.002	0.026	1.432	0.0002
Inv.3	30.0	0.060	0.016	1.452	0.280	0.246	0.0015	0.002	0.036	1.312	0.0002
Obso.1	21.5	0.048	0.060	1.871	0.404	0.360	0.0042	0.077	0.030	2.415	0.0008
Obso.2	24.0	0.045	0.030	2.054	0.228	0.424	0.0032	0.105	0.044	2.903	0.0004
Obso.3	22.9	0.033	0.022	1.878	0.152	0.374	0.0038	0.124	0.026	2.335	0.0010

Table 5.3: Summary of XRF soil data of all 11 *Polhillia* species. Parameters investigated include: Nickel (Ni), Copper (Cu), Zinc (Zn), Gallium (Ga), Germanium (Ge), Bromine (Br), Rubidium (Rb), Strontium (Sr), Yttrium (Y) and Zirconium (Zr). Brevi. = *Polhillia brevicalyx*; Conn. = *Polhillia connata*; Curt. = *Polhillia curtisiae*; Pall. = *Polhillia pallens*; Stirt. = *Polhillia stirtoniana*; Xair. = *Polhillia xairuensis*; Fort. = *Polhillia fortunata;* Groen. = *Polhillia groenewaldii*; Igno. = *Polhillia ignota*; Inv. = *Polhillia involucrata*; Obso. = *Polhillia obsoleta*.

Sample	Ni %	Cu %	Zn %	Ga %	Ge %	Br %	Rb %	Sr %	Y %	Zr %
Overberg										
Brevi.1	0.006	0.0016	0.0046	0.0010	0.0008	0.0030	0.0085	0.0084	0.0035	0.039
Brevi.2	0.006	0.0013	0.0046	0.0013	0.0007	0.0022	0.0087	0.0054	0.0027	0.032
Brevi.3	0.006	0.0017	0.0068	0.0012	0.0005	0.0029	0.0091	0.0081	0.0037	0.028
Conn.1	0.003	0.0014	0.0055	0.0013	0.0006	0.0014	0.0088	0.0058	0.0042	0.047
Conn.2	0.002	0.0015	0.0062	0.0013	0.0008	0.0026	0.0100	0.0078	0.0038	0.030
Conn.3	0.001	0.0006	0.0018	0.0011	0.0007	0.0051	0.0074	0.0094	0.0037	0.039
Curt.1	0.002	0.0020	0.0138	0.0012	0.0005	0.0036	0.0089	0.0065	0.0044	0.052
Curt.2	0.001	0.0013	0.0021	0.0016	0.0003	0.0065	0.0121	0.0137	0.0043	0.029
Curt.3	0.001	0.0010	0.0018	0.0013	0.0006	0.0051	0.0061	0.0092	0.0026	0.037
Pall.1	0.006	0.0014	0.0062	0.0014	0.0005	0.0032	0.0117	0.0084	0.0026	0.020
Pall.2	0.007	0.0021	0.0100	0.0018	0.0006	0.0041	0.0150	0.0089	0.0028	0.012
Pall.3	0.122	0.0044	0.0037	0.0013	0.0005	0.0024	0.0103	0.0069	0.0036	0.028
Stirt.1	0.111	0.0032	0.0015	0.0008	0.0003	0.0031	0.0049	0.0054	0.0028	0.030
Stirt.2	0.103	0.0039	0.0040	0.0011	0.0005	0.0036	0.0060	0.0073	0.0028	0.027
Stirt.3	0.003	0.0028	0.0094	0.0018	0.0005	0.0034	0.0152	0.0142	0.0034	0.016
Xair.1	0.005	0.0014	0.0032	0.0011	0.0004	0.0076	0.0081	0.0080	0.0033	0.022
Xair.2	0.006	0.0023	0.0044	0.0011	0.0007	0.0030	0.0092	0.0098	0.0069	0.025
Xair.3	0.006	0.0019	0.0081	0.0012	0.0009	0.0018	0.0094	0.0043	0.0033	0.021
Outside Overberg										
Fort.1	0.004	0.0027	0.0111	0.0021	0.0011	0.0018	0.0167	0.0106	0.0039	0.017
Fort.2	0.004	0.0027	0.0122	0.0018	0.0009	0.0024	0.0168	0.0101	0.0037	0.020
Fort.3	0.003	0.0023	0.0083	0.0017	0.0006	0.0005	0.0147	0.0077	0.0034	0.024
Groen.1	0.003	0.0020	0.0086	0.0018	0.0009	0.0010	0.0134	0.0094	0.0037	0.027
Groen.2	0.003	0.0020	0.0087	0.0017	0.0007	0.0007	0.0134	0.0090	0.0039	0.029
Groen.3	0.003	0.0021	0.0090	0.0019	0.0006	0.0007	0.0135	0.0089	0.0037	0.027
Igno.1	0.008	0.0030	0.0259	0.0009	0.0004	0.0053	0.0054	0.0047	0.0014	0.026
Igno.2	0.008	0.0020	0.0023	0.0005	0.0004	0.0019	0.0025	0.0035	0.0017	0.039
Igno.3	0.001	0.0008	0.0013	0.0005	0.0003	0.0014	0.0024	0.0035	0.0012	0.030
Inv.1	0.001	0.0013	0.0054	0.0010	0.0003	0.0002	0.0092	0.0084	0.0017	0.031
Inv.2	0.001	0.0008	0.0030	0.0007	0.0003	0.0003	0.0075	0.0115	0.0013	0.038
Inv.3	0.001	0.0010	0.0033	0.0007	0.0003	0.0002	0.0051	0.0100	0.0009	0.029
Obso.1	0.007	0.0014	0.0054	0.0010	0.0005	0.0024	0.0080	0.0037	0.0021	0.023
Obso.2	0.009	0.0014	0.0039	0.0011	0.0008	0.0012	0.0083	0.0030	0.0025	0.027
Obso.3	0.010	0.0013	0.0037	0.0010	0.0005	0.0011	0.0068	0.0026	0.0021	0.025

Table 5.4: Summary of XRF soil data of all 11 Polhillia species. Parameters investigated include: Niobium (Nb), Molybdenum (Mo), Cerium (Ce), Hafnium (Hf), Tantalum (Ta), Lead (Pb), Thorium (Th), Caesium (Cs), Barium (Ba) and Lanthanum (La). Brevi. = *Polhillia brevicalyx*; Conn. = *Polhillia connata*; Curt. = *Polhillia curtisiae*; Pall. = *Polhillia pallens*; Stirt. = *Polhillia stirtoniana*; Xair. = *Polhillia xairuensis*; Fort. = *Polhillia fortunata;* Groen. = *Polhillia groenewaldii*; Igno. = *Polhillia ignota*; Inv. = *Polhillia involucrata*; Obso. = *Polhillia obsoleta*.

Sample	Nb %	Mo %	Ce %	Hf %	Ta %	Pb %	Th %	Cs %	Ba %	La %
Overberg										
Brevi.1	0.0016	0.0003	0.0004	0.0011	0.00020	0.0019	0.0012	0.002	0.047	0.017
Brevi.2	0.0014	0.0003	0.0004	0.0008	0.00016	0.0018	0.0012	0.002	0.050	0.532
Brevi.3	0.0016	0.0003	0.0004	0.0010	0.00018	0.0020	0.0011	0.002	0.041	0.017
Conn.1	0.0019	0.0003	0.0291	0.0013	0.00017	0.0022	0.0016	0.002	0.041	0.017
Conn.2	0.0016	0.0003	0.0215	0.0006	0.00020	0.0019	0.0014	0.002	0.050	0.017
Conn.3	0.0016	0.0003	0.0004	0.0006	0.00015	0.0021	0.0011	0.002	0.038	0.122
Curt.1	0.0017	0.0003	0.0120	0.0013	0.00016	0.0018	0.0015	0.002	0.040	0.017
Curt.2	0.0018	0.0003	0.0232	0.0005	0.00017	0.0027	0.0018	0.002	0.044	0.901
Curt.3	0.0018	0.0003	0.0165	0.0008	0.00018	0.0021	0.0015	0.002	0.030	0.471
Pall.1	0.0017	0.0003	0.0004	0.0003	0.00017	0.0022	0.0013	0.008	0.052	0.318
Pall.2	0.0015	0.0003	0.0109	0.0008	0.00021	0.0026	0.0014	0.014	0.046	0.017
Pall.3	0.0014	0.0028	0.0163	0.0011	0.00013	0.0021	0.0013	0.002	0.050	0.473
Stirt.1	0.0014	0.0024	0.0004	0.0012	0.00015	0.0018	0.0009	0.006	0.021	0.017
Stirt.2	0.0014	0.0024	0.0004	0.0006	0.00010	0.0020	0.0011	0.002	0.028	0.017
Stirt.3	0.0018	0.0003	0.0004	0.0008	0.00018	0.0025	0.0012	0.002	0.046	0.017
Xair.1	0.0013	0.0003	0.0004	0.0007	0.00019	0.0019	0.0012	0.002	0.032	0.097
Xair.2	0.0016	0.0003	0.0205	0.0001	0.00019	0.0019	0.0013	0.009	0.050	0.426
Xair.3	0.0014	0.0003	0.0004	0.0007	0.00016	0.0020	0.0013	0.009	0.051	0.384
Outside Overberg										
Fort.1	0.0019	0.0003	0.0004	0.0004	0.00018	0.0028	0.0017	0.002	0.064	0.017
Fort.2	0.0018	0.0003	0.0239	0.0010	0.00023	0.0030	0.0019	0.008	0.060	1.046
Fort.3	0.0020	0.0003	0.0147	0.0007	0.00018	0.0022	0.0015	0.005	0.079	0.017
Groen.1	0.0021	0.0003	0.0287	0.0005	0.00014	0.0027	0.0014	0.015	0.086	0.748
Groen.2	0.0022	0.0003	0.0004	0.0008	0.00016	0.0027	0.0014	0.002	0.089	1.199
Groen.3	0.0020	0.0003	0.0004	0.0004	0.00012	0.0028	0.0014	0.029	0.085	0.663
Igno.1	0.0009	0.0003	0.0004	0.0004	0.00012	0.0034	0.0008	0.002	0.032	0.017
Igno.2	0.0009	0.0003	0.0004	0.0009	0.00013	0.0011	0.0004	0.002	0.034	0.017
Igno.3	0.0008	0.0003	0.0199	0.0002	0.00012	0.0009	0.0004	0.002	0.030	0.017
Inv.1	0.0011	0.0003	0.0004	0.0003	0.00016	0.0017	0.0008	0.002	0.078	0.017
Inv.2	0.0008	0.0003	0.0004	0.0007	0.00019	0.0013	0.0006	0.020	0.068	0.017
Inv.3	0.0006	0.0003	0.0004	0.0002	0.00016	0.0012	0.0005	0.008	0.051	0.017
Obso.1	0.0009	0.0003	0.0004	0.0005	0.00018	0.0022	0.0008	0.007	0.033	0.017
Obso.2	0.0012	0.0003	0.0156	0.0008	0.00015	0.0018	0.0009	0.002	0.036	0.017
Obso.3	0.0010	0.0003	0.0004	0.0007	0.00013	0.0016	0.0008	0.005	0.034	0.017

3.2.1. Within Overberg

Soils among species growing in the Overberg region (*P. brevicalyx*, *P. connata*, *P. curtisiae*, *P. pallens*, *P. stirtoniana* and *P. xairuensis*) were found to be fairly similar in composition, as indicated by the substantial overlap in the PCA plot (Figure 2). Significant differences in soil parameters between these six species are summarized in Tables 6.1 and 6.2. Soil pH for most species ranged between 5.6-5.9 on average, although substantial inter-population variation was seen in most species, in particular *P. brevicalyx* (pH: 5.2–6.9). Differences in soil pH between these species was largely non-significant, except for *P. stirtoniana* that grows on less acidic soils compared to *P. pallens* and *P. xairuensis*. Resistance varied substantially between populations of species thus obscuring possible patterns, with only *P. xairuensis* found to have significantly higher soil resistance. Soil composition fractions were variable, but few patterns were detectable due to substantial inter-population variation. Percentage sand ranged between 57% and 83%, while silt and clay ranged between 8–26% and 9–17%, respectively. Only *P. curtisiae* was found to have significantly higher sand fractions than *P. pallens* and *P. xairuensis*, while the inverse pattern was seen for silt fraction. No significant difference was found for clay fraction between Overberg species. Soil NH_4 was, for the most part, not significantly different between Overberg species and substantial inter-population variation was also prevalent. Only *P. connata* was found to have significantly lower NH_4 compared to *P. pallens*. Total cation (T-value) soil concentrations were largely similar between species, and were only significantly higher in soils of *P. curtisiae* and *P. pallens* compared to *P. connata*. Concentrations of macro elements differed between Overberg species with Al in particular having significantly higher concentrations in soils of *P. connata* and *P. curtisiae*. Soil Si concentrations were found to be significantly higher in soils of *P. connata* and even more so in those of *P. xairuensis*. Soil Na varied substantially across the Overberg region, but no significant patterns were found due to substantial inter-population variation.

Table 6.1: Summary of significant parameter differences among the six Overberg *Polhillia* species. Letters under each soil parameter indicate species with significantly lower concentrations of that soil parameter compared to other species (A to F) as numbered in column 1. Significance values set at $p<0.05$ and number of asterisks (*) indicate zeros following the p-value decimal place to a maximum of three zero decimals.

Species	pH	Resistance	Sand	Silt	NH_4	T-value	Mg	Al	Si	K	Ca	Y	V
brevicalyx **(A)**													
connata **(B)**								E* F*	D* E*				
curtisiae **(C)**			D* F*			B*		A* E** F**			F*		B*
pallens **(D)**				C**	B*	B*	A* C*	F*		E*	F*		B*
stirtoniana **(E)**	D* F*												
xairuensis **(F)**		C* D* E*		C*					D** E**			D* E*	

Table 6.2: Summary of significant parameter differences among the six Overberg *Polhillia* species. Letters under each soil parameter indicate species with significantly lower concentrations of that soil parameter compared to other species (A to F) as numbered in column 1. Significance values set at $p<0.05$ and number of asterisks (*) indicate zeros following the p-value decimal place to a maximum of three zero decimals.

Species	Cr	Mo	Mn	Fe	Co	Ni	Cu	Ti	Zr	Nb	Th	Ba	Ge	Ce
brevicalyx **(A)**									D*			E*		
connata **(B)**								E**	D** E* F**	F**	E*		C* E*	
curtisiae **(C)**								E**	D** E** F**	F*	A** E** F*			A* E*
pallens **(D)**			C*	A* F**	B* C*		A* B* C*					E*		
stirtoniana **(E)**	A** B** C** F**	A** B** C** F**	B** C**	A* F**	B* C*	A** B** C** F**	A** B*** C** F**							
xairuensis **(F)**														

Patterns of trace element concentrations among soils of Overberg species proved interesting. Soils of *P. pallens,* and more significantly *P. stirtoniana,* were richer in metals such as Cr, Mo, Mn, Fe, Co, Ni and Cu. Soils of *P. connata* and *P. curtisiae*, however, had significantly higher concentrations of Ti, Zr, Nb and Th. No statistical difference in concentrations of soil P, S, Zn, Ga, Ge, Br, Rb, Sr, Cs, La, Hf and Pb were found among Overberg species. Overall, it appears that the soils of *P. pallens* and *P. stirtoniana* were substantially different from that of all other species in the region and most similar to one another. The soils of *P. connata* and *P. curtisiae* also appeared to be distinct within the region and were similar to one another. *Polhillia brevicalyx* soils rarely had any significantly different (unique) parameter measurements, but appeared to be most similar to *P. connata* soils. *Polhillia xairuensis* soils

appeared to be distinct as they mostly had significantly lower concentrations of the metals than the high metal concentrations in soils of *P. connata*, *P. curtisiae*, *P. pallens* and *P. stirtoniana*.

3.2.2. Outside Overberg

The composition of soils of species growing outside of the Overberg region (*P. groenewaldii*, *P. fortunata*, *P. ignota*, *P. involucrata* and *P. obsoleta*) differed substantially (Table 7.1–7.3). Soil pH was significantly different between these species, with *P. obsoleta* and *P. ignota* growing in relatively acidic soils (pH: 4.8–5.5 and 4.9–5.3, respectively). *Polhillia fortunata* appears to favour more alkaline soils with a pH of 7–7.4, while a soil pH of 7.9 was found at the *P. groenewaldii* site, the most alkaline soil of all *Polhillia* species. Soil pH varied substantially between *P. involucrata* populations, ranging from 4.7 to 7.1. Resistance varied substantially between different populations of the same species, but *P. involucrata* had significantly higher mean soil resistance than other non-Overberg species. Soil composition fractions were consistent between populations of the various species, allowing for distinct patterns to be observed. Percentage sand was significantly lower in the soils of *P. fortunata* (41%) than in soils of the other species (71% to 89%). Percentage silt was significantly higher in soils of *P. fortunata* (40%), compared to 4–12% silt in the other non-Overberg species. Soil clay percentages between the five species were more similar, but *P. fortunata* still had significantly higher clay fractions (19%) compared to other species (7–17%). Soils of *P. involucrata* were found to be deficient in NH_4 and had significantly lower concentrations compared to most *Polhillia* species, both within and outside of the Overberg. Total cation (T-value) soil concentrations were significantly higher in soils of *P. groenewaldii* and *P. fortunata* compared to other non-Overberg species. Concentrations of macro elements differed substantially between non-Overberg species, showing some interesting patterns. Soil Na concentrations were very high in soils of *P. fortunata* and were significantly higher than in soils of all other *Polhillia* species. Soils of *P. ignota* were deficient in Mg, with significantly lower concentrations than in the soils of all other non-Overberg species, except *P. involucrata*. Soils of *P. groenewaldii* and *P. fortunata* had significantly higher concentrations of Al and K compared to other species, especially *P. ignota*. Soils of *P. involucrata* had the highest concentrations of P and Si among non-Overberg species, but were only found to be significantly higher compared to *P. obsoleta* (and *P. groenewaldii* for P only). Soil S concentrations were significantly higher in soils of *P.*

ignota compared to other non-Overberg species, although substantial inter-population variation in S was observed.

Table 7.1: Summary of significant parameter differences among the five non-Overberg *Polhillia* species. Letters under each soil parameter indicate species with significantly lower concentrations of that soil parameter compared to other species (A to E) as numbered in column 1. Significance values set at $p<0.05$ and number of asterisks (*) indicate zeros following the p-value decimal place to a maximum of three zero decimals. P-values with greater than three zero-decimals are indicated in bold text.

Species	pH	Resistance	Sand	Silt	Clay	NH_4	T-value	Na	Mg
***groenewaldii* (A)**	**C***** D*** **E*****		**B*****	C**		D*	C** **D***** E***		C*** D* E*
***fortunata* (B)**	C*** D* E***			**A***** **C***** **D***** **E*****	A** **C***** D** E*	D*	C** **D***** E***	A** C*** D** E***	C**
***ignota* (C)**			A* **B***** E*			D**			
***involucrata* (D)**		A** B** C** E**	**B*****						
***obsoleta* (E)**			**B*****		C*				C*

Table 7.2: Summary of significant parameter differences among the five non-Overberg *Polhillia* species. Letters under each soil parameter indicate species with significantly lower concentrations of that soil parameter compared to other species (A to E) as numbered in column 1. Significance values set at $p<0.05$ and number of asterisks (*) indicate zeros following the p-value decimal place to a maximum of three zero decimals. P-values with greater than three zero-decimals are indicated in bold text.

Species	Al	Si	P	S	K	Y	V	Fe	Ga	Rb	Sr
groenewaldii **(A)**	C*** D* E*				C***	C** D*** E*	C** D*	C*** D*** E*	**C***** **D***** E****	**C***** D** E**	C** E**
fortunata **(B)**	C*** D* E*				C***	C** D** E*	A* **C***** **D***** E**	C*** **D***** E***	**C***** **D***** **E*****	**C***** D*** E***	C** E**
ignota **(C)**				A*** B** D*** E**							
involucrata **(D)**		E**	A* E*		C*						C** E**
obsoleta **(E)**	C*				C*		C*	C*		C*	

Table 7.3: Summary of significant parameter differences among the five non-Overberg *Polhillia* species. Letters under each soil parameter indicate species with significantly lower concentrations of that soil parameter compared to other species (A to E) as numbered in column 1. Significance values set at $p<0.05$ and number of asterisks (*) indicate zeros following the p-value decimal place to a maximum of three zero decimals. P-values with greater than three zero-decimals are indicated in bold text.

Species	Cu	Ti	Zr	Nb	Th	Br	Ba	Ge	Ta	Cs	La	Pb
groenewaldii **(A)**		C* D*** E**		**C***** **D***** **E*****	**C***** **D***** E***		B** **C***** D** **E*****	C** D***		B* C** E*	B* C*** D*** E***	C* D**
fortunata **(B)**	D* E*	C** D*** E**		**C***** **D***** **E*****	A* **C***** **D***** **E*****		**C***** **E*****	C*** **D***** E*	A* C** E*			D**
ignota **(C)**			B*			D*						
involucrata **(D)**			B*				**C***** **E*****					
obsoleta **(E)**					C*			D**				

Soils of *P. groenewaldii* and especially *P. fortunata* had significantly higher concentrations of trace elements compared to the soils of *P. ignota*, *P. involucrata* and *P. obsoleta* (Table 7.2 and 7.3). These include Y, V, Fe, Ga, Rb, Sr, Ti, Nb, Th, Ba, and Ge. Soils of *P. groenewaldii* also had significantly higher concentrations of Cs and La compared to soils of other non-Overberg species. Significantly higher Zr concentrations were found in soils of *P. ignota* and *P. involucrata* compared to *P. fortunata*. Soil Ta was significantly higher in *P. fortunata* soils than in soils of other non-Overberg species, except *P. involucrata*. No statistical difference in the concentrations of soil Ca, Cr, Mn, Co, Ni, Zn, Mo, Ce and Hf were found between non-Overberg species.

Overall, it appears that soils of *P. groenewaldii* and *P. fortunata* are substantially different to those of all other non-Overberg species due to the high concentrations of trace metals and high soil pH levels. The soils of these two species are relatively similar to one another, but *P. fortunata* has a significantly higher silt and lower sand fraction than *P. groenewaldii*, and *vice versa*. The soils of *P. ignota*, *P. involucrata* and *P. obsoleta* are distinct, but rather similar to one another, with a few important exceptions. *Polhillia involucrata* soils had significantly

higher resistance, lower NH_4 and higher P concentrations. *Polhillia ignota* and *P. obsoleta* soils appear to be similar, but *P. obsoleta* had a significantly higher clay fraction and higher concentrations of Mg as well as several trace metals. Soils of *P. ignota* had a significantly higher sand fraction and higher concentrations of S.

3.2.3. PCA analysis

The PCA (Figure 2) showed that some *Polhillia* species have very distinctive soil profiles, while others appear to have soil profiles that overlap substantially. *Polhillia pallens* and *P. stirtoniana* both grow in variable soils, although most localities are characterized by significantly higher levels of metals including Cr, Mo, Mn, Co, Cu and Ni. There is also substantial overlap in soil profiles of *P. brevicalyx*, *P. connata*, *P. curtisiae* and *P. xairuensis* (the remaining Overberg species), with very few differences in their soil profiles. *Polhillia brevicalyx* and *P. connata* appear to occupy a fairly narrow range of soil profile variability. It is important to note, though, that these species do not have overlapping distributions.

Non-Overberg species soils are all distinct from one another (Figure 2), but can be divided into two groups. Soils of *P. fortunata* and *P. groenewaldii* are strongly correlated with high concentrations of cations and metals, soil pH of 7–7.9 and lower concentrations of sand, while soils of *P. ignota*, *P. involucrata* and *P. obsoleta* are correlated with generally low soil pH, high sand concentrations and soils with significantly lower concentrations of cations and metals. Within this group, high resistance, P and Si concentrations characterize *P. involucrata* soils, while *P. ignota* soils have the lowest concentrations of metals and cations and the highest sand fractions. *Polhillia fortunata* soils are distinct from *P. groenewaldii* in Figure 2 based primarily on having higher silt and clay fractions. Figure 2 also visualizes the distinction in soils between sister species *P. groenewaldii* and *P. obsoleta*. It is also interesting to note that Overberg and non-Overberg soils appear to be relatively distinct from one another, even though non-Overberg soils form two distinct groupings.

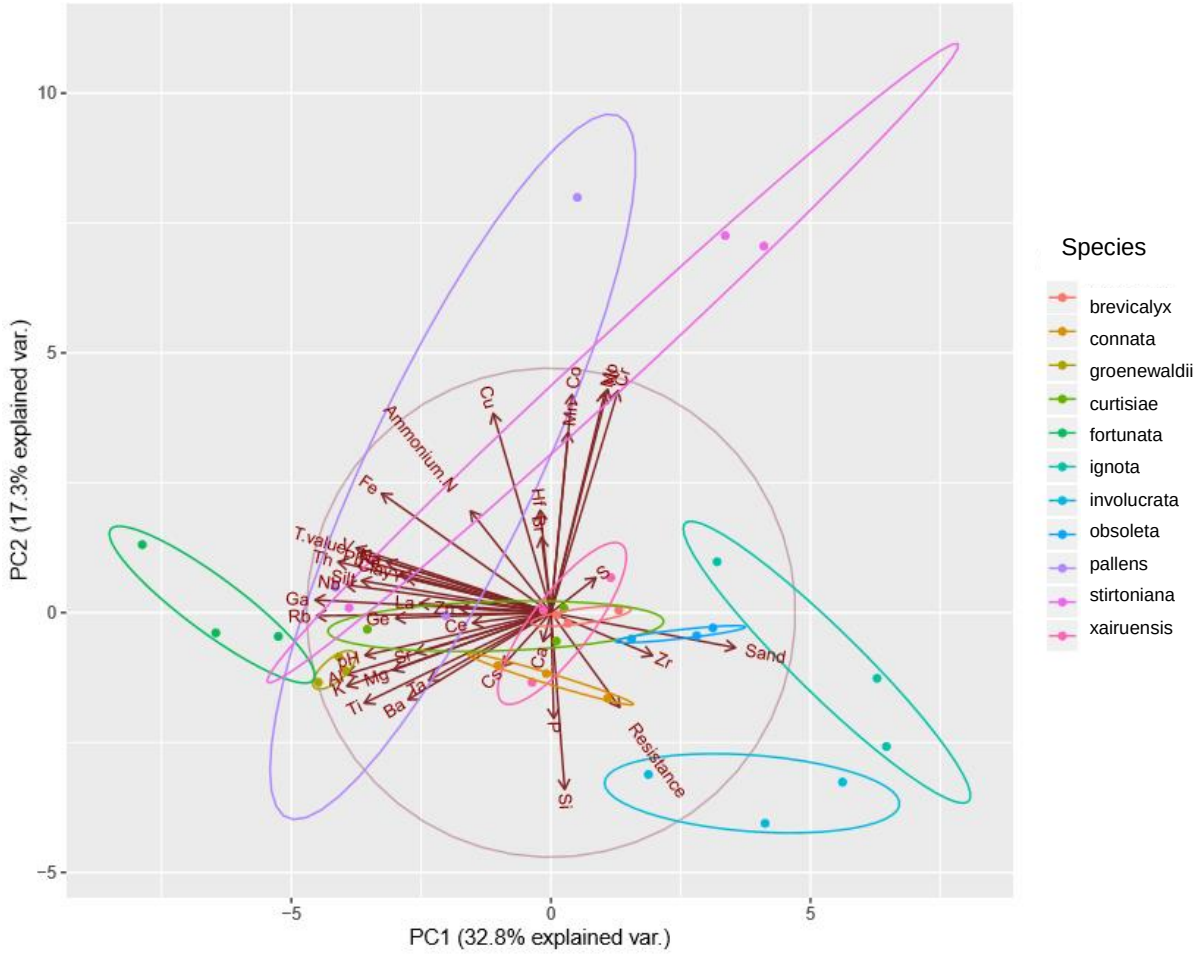

Figure 2: Principal Component Analysis (PCA) plot representing soil components that group soil characters for all 11 *Polhillia* species. The soil data used in this analysis correspond to those summarized in Table 5.1–5.4 above.

3.3. Rhizobia

Rhizobia were successfully isolated from nodules of eight of the nine species cultivated under greenhouse conditions. Nodules harvested from *Polhillia* species were generally small (less than 1 mm long in some cases), round to oblong, and in many cases, plants were found to have few or no rhizobia (Figure 3). *Polhillia involucrata* had numerous nodules that were substantially larger than those harvested from other species. Nodules of *P. connata*, *P. obsoleta* and *P. stirtoniana* were comparable in size to those of *P. involucrata* (2–4 mm), but were less abundant on roots. Nodules of the remaining four species were minute and occurred in low densities on roots.

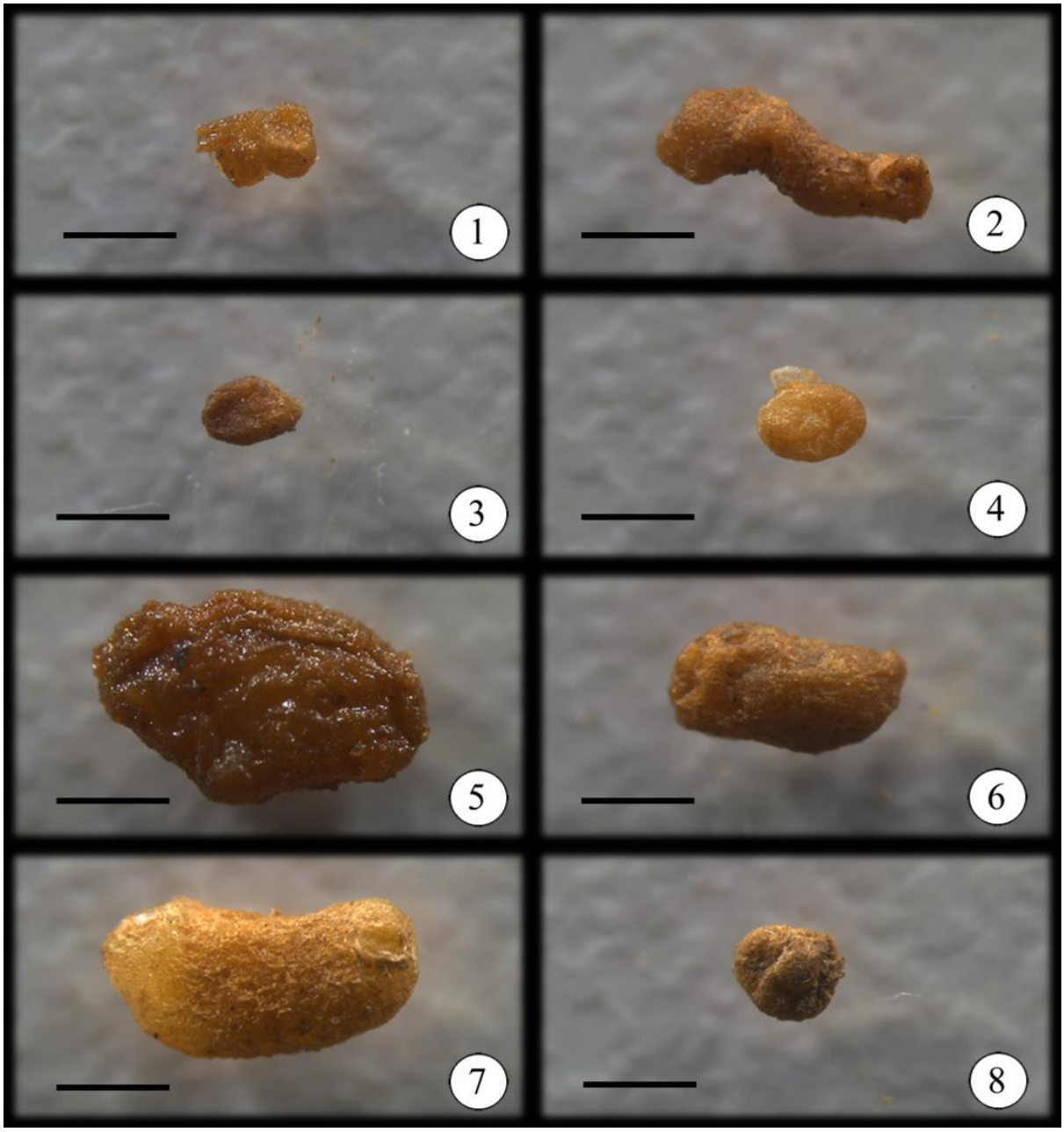

Figure 3: Nodules harvested from *Polhillia* species grown in native soil in a greenhouse for 5 months. 1 – *P. brevicalyx*; 2 – *P. connata*; 3 – *P. curtisiae*; 4 – *P. ignota*; 5 – *P. involucrata*; 6 – *P. obsoleta*; 7 – *P. stirtoniana*; 8 – *P. xairuensis*. Scale bars: 1 mm.

GenBank nucleotide BLAST analysis revealed that two rhizobial genera nodulate *Polhillia* species (Table 8). Both 16S and recA regions retrieved *Mesorhizobium* taxa from nodules harvested from *P. connata*, *P. curtisiae*, *P. ignota*, *P. obsoleta* and *P. stirtoniana*, while *Rhizobium* was isolated from nodules harvested from *P. brevicalyx*, *P. involucrata* and *P. xairuensis*. The 16S BLAST analysis placed most *Mesorhizobium* isolates as being 99-100%

similar to *Mesorhizobium* sp. strain WSM3267, while the symbiont of *P. ignota* matched to 100% with *Mesorhizobium* sp. MM5361. *Rhizobium* isolates also were placed as 100% or 99% similar to *Rhizobium leguminosarum* strain S11190.

Table 8: BLASTn search results for *Polhillia* rhizobial symbionts isolated from seedlings grown under greenhouse conditions in native soils. Percentage similarities to the closest sequences on GenBank are shown.

Region	Host	Closest match	GenBank Accession	Similarity (%)
16S rRNA	*P. brevicalyx*	*Rhizobium leguminosarum* strain S11190	MF977616.1	100
	P. connata	*Mesorhizobium* sp. strain WSM3267	MF949017.1	99
	P. curtisiae	*Mesorhizobium* sp. strain WSM3267	MF949017.1	100
	P. ignota	*Mesorhizobium* sp. MM5361	KF802569.1	100
	P. involucrata	*Rhizobium leguminosarum* strain S11190	MF977616.1	100
	P. obsoleta	*Mesorhizobium* sp. strain WSM3267	MF949017.1	100
	P. stirtoniana	*Mesorhizobium* sp. strain WSM3267	MF949017.1	100
	P. xairuensis	*Rhizobium leguminosarum* strain S11190	MF977616.1	100
recA	*P. brevicalyx*	*Rhizobium* sp. RPVR04	GQ863532.1	97
	P. connata	*Mesorhizobium* sp. BL34-R1	KM188327.1	97
	P. curtisiae	*Mesorhizobium* sp. BL34-R1	KM188327.1	98
	P. ignota	*Mesorhizobium australicum* LMG24608T	FR863559.1	99
	P. involucrata	*Rhizobium* sp. RPVR04	GQ863532.1	95
	P. obsoleta	*Mesorhizobium* sp. MM5333	KF802780.1	100
	P. stirtoniana	*Mesorhizobium* sp. BL34-R1	KM188327.1	97
	P. xairuensis	*Rhizobium* sp. strain NAK 288	MG288726.1	97

The recA BLAST analysis, however, showed that there may be multiple rhizobial symbiont lineages. *Polhillia connata*, *P. stirtoniana* and *P. curtisiae* isolates were 97% and 98% similar to *Mesorhizobium* sp. BL34-R1, while the *P. ignota* isolate was 99% similar to *Mesorhizobium australicum* LMG24608T and the isolate of *P. obsoleta* 100% similar to *Mesorhizobium* sp. MM5333. Rhizobium isolates also varied with *P. brevicalyx* and *P. involucrata* isolates being 97% and 95% similar to *Rhizobium* sp. RPVR04, while the *P. xairuensis* isolate was 97% similar to *Rhizobium* sp. strain NAK 288.

In the phylogenetic reconstruction of the 16S region (Figure 4), *P. brevicalyx*, *P. involucrata* and *P. xairuensis* symbionts resolve along with *Rhizobium leguminosarum* strain S11190 and two other isolates as a single clade sister to the *Rhizobium* sp. strain N9 isolate. This clade is also a strongly supported lineage differing from the other lineage comprised of *Rhizobium* sp. OD49 and *Rhizobium* sp. OD123 that were isolates from other Cape legumes. The clade containing *Mesorhizobium* isolates is largely unresolved, only supporting a clade containing isolates of *P. connata* and *P. ignota* with 0.59 posterior probability. Details of outgroup used in this phylogeny are summarized in Table 2.

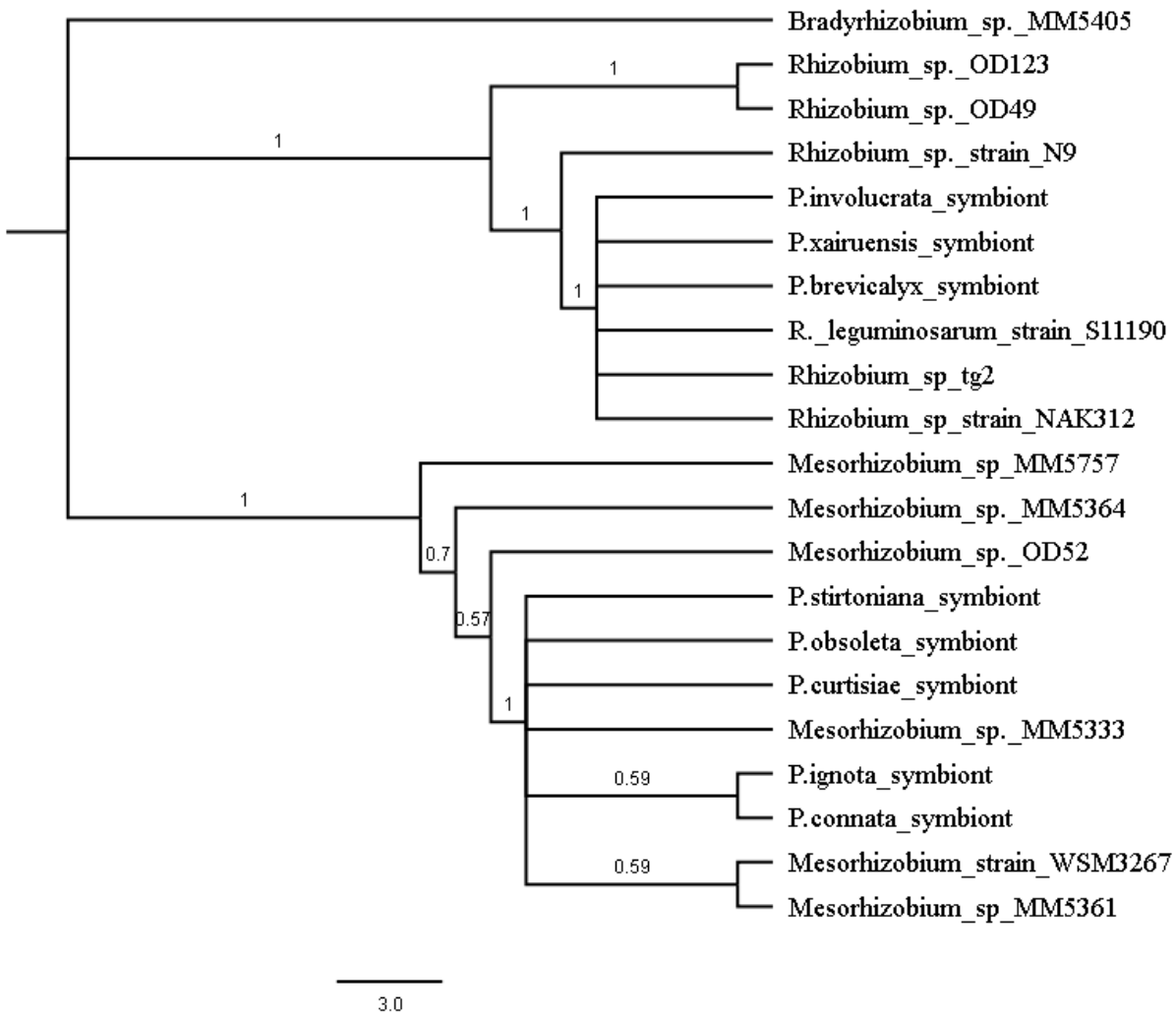

Figure 4: Phylogenetic tree of the 16S rRNA region produced through Bayesian inference showing rhizobial symbiont patterns among eight *Polhillia* species. *Polhillia* symbiont isolates are highlighted in bold text. Values above branches represent posterior probabilities.

The recA phylogenetic tree (Figure 5) was better resolved than the 16S tree and showed that the *Rhizobium* isolates from *P. brevicalyx*, *P. involucrata* and *P. xairuensis* to form a monophyletic lineage with 0.81 probability, grouping with no outgroup isolates. This lineage is also clearly distinct from *Rhizobium leguminosarum* (Frank 1879) Frank (1889) with which isolates matched with up to 100% similarity in the 16S BLAST analysis (Table 8). *Mesorhizobium* isolates of *P. ignota* and *P. obsoleta* each formed distinct lineages grouping with *Mesorhizobium australicum* Nandasena *et al.* (2009) and *Mesorhizobium* sp. MM5333, respectively. The *Mesorhizobium* isolates of *P. connata*, *P. curtisiae* and *P. stirtoniana* also form a distinct monophyletic group with 71% probability, which also does not group with any outgroup isolates. Details of outgroup used in this phylogeny are summarized in Table 3.

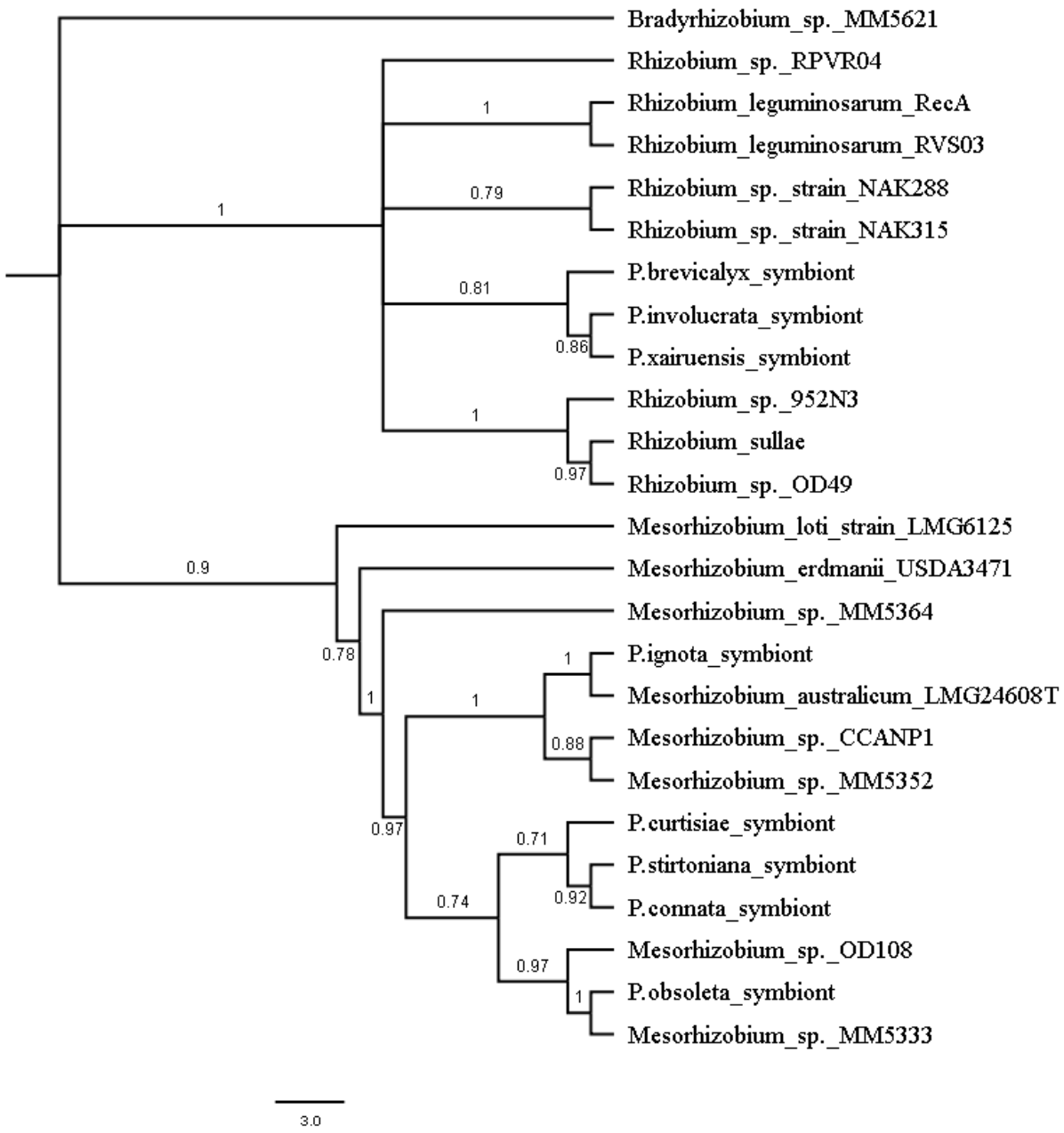

Figure 5: Phylogenetic tree of the recA region produced through Bayesian inference showing rhizobial symbiont patterns among eight *Polhillia* species. *Polhillia* symbiont isolates are highlighted in bold text. Values above branches represent posterior probabilities.

4. DISCUSSION

4.1. Habitat

Polhillia species distributions generally do not overlap, and most species are geographically distinct, especially the non-Overberg species. In the Overberg, six species occur within a relatively small area, yet species distributions only overlap between *P. stirtoniana* and *P. curtisiae*, and to some extent between *P. stirtoniana* and *P. brevicalyx*. All these species occur in Renosterveld vegetation, but species appear to favour certain landscape topographies that maintain allopatry even where distributions overlap. The underlying geology differed between some species, while *P. xairuensis* and *P. curtisiae* occur on both shale and silcrete/ferricrete. The changes in underlying geology were thought to be provide edaphic differences, but Cowling and Holmes (1992) report that species rarely occur on only shale or only silcrete/ferricrete, as these soils are relatively similar, because silcrete/ferricrete usually overlay clay in the Overberg (Thwaites and Cowling, 1988). Several populations of *P. stirtoniana* and *P. curtisiae*, for example, are within a few hundred metres of each other. However, *P. stirtoniana* consistently prefer growing in gullies and south facing slopes, while *P. curtisiae* consistently prefer gentle to moderate north and west facing hill slopes. *Polhillia brevicalyx* grows only along a single river course, while *P. stirtoniana* is restricted to nearby gullies. It is important to note that in the context of *Polhillia*, these three species are distantly related to one another according to the molecular phylogeny of du Preez *et al*. (2019 – Chapter 2 and 3). Putatively closely related species such as *P. curtisiae*, *P. pallens* and *P. xairuensis* are geographically distinct, but also have very different habitat preferences. More distantly related species such as *P. connata*, *P. stirtoniana* and *P. xairuensis* are geographically distinct, but often occupy similar habitats, especially in terms of landscape topological preference. Differences in interspecific habitat preference therefore appear to play an important role in maintaining species allopatry where distributions overlap, and along with geographical isolation, are important in separating putatively closely related species. This is, however, not important for distantly related and geographically distinct species as they do not compete for similar niches.

Non-Overberg *Polhillia* species are all geographically very distinct from one another, and differ somewhat in their habitat preferences. *Polhillia involucrata* is the odd species in the genus (du Preez *et al*., 2019 – Chapter 2 and 3), and this is also reflected in its habitat preference. It is the only species that occurs outside of the GCFR and at altitudes of 1000–1400 m above sea level, while all other species grow in lowlands no higher than 350 m above

sea level. Although rainfall data were not included in our analyses, it is also the only species to occur in a summer rainfall region, compared to winter-rainfall of all other species (Manning and Goldblatt, 2012; Snijman, 2013). Three closely related species, *P. groenewaldii*, *P. ignota* and *P. obsoleta*, are morphologically difficult to separate (du Preez *et al*., 2019 – Chapter 3), but differ in that *P. groenewaldii* grows on a shale hillslope, while the latter two species grow in alluvial sediments on flats or slopes. *Polhillia groenewaldii* and *P. fortunata* grow in similar landscape topologies and on shale, but are geographically and morphologically very distinct (du Preez *et al*., 2019 – Chapter 3). Habitat preference therefore appears to be less important for non-Overberg species as they are already geographically distinct, although a shift in preference between alluvial sediment and shale may explain the speciation of *P. obsoleta* and *P. groenewaldii.* Closely related species rarely co-occur or share similar habitats due to niche conservatism (Linder and Vlok, 1991; Wiens and Graham, 2005; Kozak and Wiens, 2006; Slingsby and Verboom, 2006). This is a likely explanation why *Polhillia* species do not occur in sympatry, and may also explain patterns of speciation, especially within the Overberg region.

4.2. Soil Analysis

Analysis of various soil parameters including pH, resistance, sand; silt; clay fractions, NH_4 concentrations as well as concentrations of macro and trace elements were conducted to identify patterns of potential preference to certain soils. Of all 11 *Polhillia* species, *P. pallens* and *P. stirtoniana* appear to grow in the most variable soils, while species such as *P. connata*, *P. brevicalyx* and *P. obsoleta* appear to occupy very specific soil profiles based on the visualization of the PCA plot (Figure 2). Within the Overberg, we found that that both *P. stirtoniana* and *P. pallens* grow on variable soils that are characterized by having significantly higher levels of metals such as Mo, Co, Cr, Ni, Cu and Mn. These two species may have thus developed a tolerance to higher concentrations of these elements allowing them to grow where other Overberg *Polhillia* species cannot (Bringezu *et al.*, 1999). Although Dludlu *et al.* (2018a) did not highlight any of these elements as important in their study, they may be important in terms of individual species tolerance to concentrations of these metals in the soil (Bringezu *et al.*, 1999). Soils of *P. connata*, *P. curtisiae*, *P. brevicalyx* and *P. xairuensis* show less variation between collection sites, indicating possible specificity to certain soil profiles, but these species generally occupy relatively similar soils. Parameter differences between these soils were mostly restricted to trace elements, while substantial inter-population variation seen in many parameters obscured patterns and possibly indicate

that these species distributions are not influenced by soil preferences. This is not completely unexpected, as this is geographically not a large area. The underlying geology varies between shale or silcrete/ferricrete, but species are rarely restricted to only one of these geologies as the silcrete/ferricrete overlay clay (Thwaites and Cowling, 1988; Cowling and Holmes, 1992). Soils of *P. connata* and *P. curtisiae* were characterized by high concentrations of Al, Zr and Th. Although Al was not highlighted as important in Cape Peninsula legume distributions (Dludlu *et al.*, 2018a), it is recognized as a heavy metal that can influence the distribution of plant species based on species tolerance to increasing Al concentrations (Bringezu *et al.*, 1999; Ma *et al.*, 2001). There is hardly any relation between soil preferences and the *Polhillia* phylogeny, as unrelated species share similar soils, and closely related species often occur on fairly different soils. *Polhillia pallens* and *P. stirtoniana* are not closely related, but geographically distinct, yet they share similar soils. The same pattern is seen between *P. connata* and *P. curtisiae*. Putatively closely related species such as *P. curtisiae, P. pallens* and *P. xairuensis* are geographically distinct and grow in substantially different soils. Previous authors have also found that closely related species often grow on different soils (Rourke, 1972; Kurzweil *et al.*, 1991). For species with overlapping distributions, such as *P. stirtoniana* with *P. brevicalyx* and *P. curtisiae* respectively, soils between species were substantially different, which along with different habitat preference may have resulted in these species remaining in allopatry. This supports previous studies that found that sympatric species are rarely closely related (Slingsby and Verboom, 2006).

Soils of non-Overberg *Polhillia* species were far more variable, which could be explained by geographic distinctiveness and underlying geology of each of the five species. Of these species, *P. involucrata* appears to have the most variable soil profiles in terms of pH, resistance and concentrations of soil elements. Although soil of *P. groenewaldii* and *P. obsoleta* were only collected from a single population, variation between samples were minimal and based on their extremely restricted distributions, it is likely that these species have very specific soil preferences. Soils preference patterns of non-Overberg species differ from Overberg species in relation to the *Polhillia* phylogeny, as unrelated and closely related species often occur on substantially different soils. *Polhillia groenewaldii* and *P. obsoleta* are very closely related species and occur on very different soils in terms of concentrations of cations and metals such as Fe and soil pH. Dludlu *et al.* (2018a) found that Fe was one of the most important drivers of edaphic preference on the Cape Peninsula as it is toxic to plants in high concentrations (Bringezu *et al.*, 1999), while differences in soil pH are also reported as

important drivers of species distribution (Amacher *et al.*, 2007; Wagner *et al.*, 2016). It is plausible that speciation between these closely related species may have been due to distinct edaphic preferences and geographic isolation, as has been found in other Cape floral taxa (Rourke, 1972; Kurzweil *et al.*, 1991; Linder and Vlok, 1991). *Polhillia obsoleta* and *P. ignota* are also closely related species that are geographically very distinct, that have fairly different soils, but much less different than when comparing *P. obsoleta* and *P. groenewaldii* soils. The main differences between soils of these two species are that *P. obsoleta* has significantly higher Mg and significantly lower S concentrations than *P. ignota*, with both these elements being important in driving plants species distributions (Richards *et al.*, 1997; Dludlu *et al.*, 2018a). *Polhillia fortunata* is morphologically distinctive and relatively distantly related to *P. ignota* and also occupies very different soils to all other *Polhillia* species. Along with *P. groenewaldii* which is also a distant relative according to the molecular phylogeny, it is the only species to consistently grow in soils with a pH of 7 or higher, with high concentrations of cations and metals such as Fe which is toxic in high concentrations (Bringezu *et al.*, 1999). It is unique in having significantly higher silt and clay fractions, and thereby a significantly lower sand fraction as well as very high Na concentrations. These are important factors that drive plant species distribution, and the high Na levels indicate a possible tolerance to high concentrations of this element as concentrations are up to ten times higher than in soils of some other *Polhillia* species (Richards *et al.*, 1997; Blumwald, 2000; Dludlu *et al.*, 2018a). Although distantly related and geographically distinct, they also have relatively different soils based primarily on different concentrations of certain trace metals, other than the high Na, silt and clay concentrations discussed above. Non-Overberg *Polhillia* soils therefore show no relation to the *Polhillia* phylogeny as all species grow on distinct soils, no matter whether species are closely related or not. The speciation of these species can thus likely be ascribed to both edaphic factors and geographical isolation.

Overall, *Polhillia* species soils varied substantially among non-Overberg species and to a lesser extent between Overberg species. Differences among Overberg species soils were largely restricted to concentrations of trace elements. Dludlu *et al.* (2018a) found that legume distributions on the Cape Peninsula were attributed to different concentrations of clay, K, S, Fe and Zn, but we found little to no significant differences in concentrations of these components in soils of Overberg species. Only Fe was highlighted as being higher in soils of *P. pallens* and *P. stirtoniana* in this region. Non-Overberg species had more variability in

important parameters including the concentrations of clay, K, S, and Fe between soils of the different species (Dludlu *et al.*, 2018a), but this would not affect their distributions as they are already geographically distinct. Soil N and P concentrations have also been reported as important in influencing the distribution of plant species and vegetation community boundaries (Witkowski and Mitchell, 1987; Thwaites and Cowling, 1988; Richards *et al.*, 1997), but these were overall mostly not significantly different between *Polhillia* species soils. Only *P. involucrata* had significantly lower N and higher P, but this is the most geographically isolated *Polhillia* species and grows in very different climatic and edaphic conditions. Concentrations of Ca, Mg and Na are also important to plants (Richards *et al.*, 1997), these elements too were not significantly different among Overberg *Polhillia* species, while significantly higher Na concentrations were seen in soils of *P. groenewaldii*, and even more so, in *P. fortunata* outside of the Overberg. Soil pH also affects the distribution of plant species across the landscape (Amacher *et al.*, 2007; Wagner *et al.*, 2016), but once again significant differences in pH were largely reserved for non-Overberg *Polhillia* species.

Edaphic heterogeneity has been proposed as a major driver of plant speciation within the GCFR (Kurzweil *et al.*, 1991; Linder and Vlok, 1991; Cowling and Holmes, 1992; Linder, 2003). For example, Kurzweil *et al.* (1991) found that Coryciinae orchids rarely speciate in allopatry, and rather see most speciation taking place in parapatry under different edaphic conditions. This is not always the case though, for example in lineages in which attributes such as pollinator preferences, general habitat and fire-survival strategy shifts appear to have acted as drivers of speciation (Johnson, 1996; Johnson *et al.*, 1998; van der Niet and Johnson, 2009). Edaphic preferences do appear to be important in *Polhillia*. It appears to be a likely driver of *Polhillia* speciation, particularly in the Overberg region as our results show that soils differ fairly substantially between closely related species, and we can likely rule out pollinator shift due to a lack of flower morphological variation (du Preez *et al.*, 2019 – Chapter 3). Linder and Vlok (1991) found that climatic niches played an important role in the speciation of *Rhodocoma* Nees that occur geographically in close proximity to one another, but we can rule this out as well as this is typically associated with strong altitudinal and rainfall gradients that are not seen in areas such as the Overberg. We therefore suggest that speciation of *Polhillia* species within the Overberg region was driven by edaphic factors rather than geographical isolation, in line with work presented by many other authors (Kurzweil *et al.*, 1991; Linder and Vlok, 1991; Cowling and Holmes, 1992; Linder, 2003).

In the light of finding insignificant differences in proposed important soil properties among *Polhillia* species, especially in the Overberg, further testing is needed on the influence of trace elements on species distributions. Common garden experiments using field-collected soil could also be used to determine whether various species are able to grow and persist in the soils of other species. This has previously been done by Moiloa (2016) for species of *Wiborgia* Thunb. who found that species grew with varying levels of success in their respective native and non-native soils. Their long-term persistence on non-native soils was, however, not determined. Wider and more intensive sampling of soils are also needed to determine if there are perhaps soil character patterns that could better determine levels of soil preference.

4.3. Rhizobia

Rhizobial symbiont patterns were surprising. Two different genera were found to nodulate the eight *Polhillia* species studied. Promiscuity in Cape legumes is not uncommon with tribes such as the Psoraleeae, Crotalarieae and Indigofereae nodulating with multiple rhizobia genera (Lemaire *et al.*, 2015). Nodulation among Cape Genisteae are poorly studied and only three *Argyrolobium* species have been investigated thus far. They were all found to nodulate only with *Mesorhizobium* species (Lemaire *et al.*, 2015). *Argyrolobium* do, however, nodulate with *Bradyrhizobium* on the SA Great Escarpment (Beukes *et al.*, 2016), although this is considered as a different geographical region. *Bradyrhizobium* was also found to nodulate with various Genisteae taxa in the Mediterranean region (Vinuesa *et al.*, 2005; Bourebaba *et al.*, 2016), which is more similar to the Cape than the SA Great Escarpment. The preference of the Genisteae for *α*-proteobacteria is interesting as *Burkholderia* (a *β*-proteobacterium) is a prominent symbiont among most Cape legumes outside of Genisteae (Howieson *et al.*, 2013; Lemaire *et al.*, 2015; Dludlu *et al.*, 2018b). To truly test Genisteae taxa's preference for *α*-proteobacteria, wider sampling of SA Genisteae taxa within the GCFR and the summer rainfall region is required. This is especially important as *Melolobium* and *Dichilus* symbionts have not been studied and *Argyrolobium* has not been sampled in different habitats.

This study represents the first broad-scale rhizobial investigation in Cape Genisteae and further illustrates the diversity of rhizobial symbionts that exists among Cape legumes. The three *Polhillia* species nodulating with *Rhizobium* are both geographically and phylogenetically distinct from one another, but appear to share a potentially novel symbiont. This is strange as rhizobial differentiation can be linked to site elevation (Lemaire *et al.*,

2015) and these two *Polhillia* species differ in site elevation by 1200 m. Another possible reason for sharing of symbionts is that the habitats are edaphically similar, but we can rule this out as our soil analysis showed that the soils of *P. involucrata* are substantially different to those of *P. brevicalyx* and *P. xairuensis* in terms of having lower concentrations of cations, metals and NH_4, higher soil P concentrations, as well as higher resistance and sand fractions. *Polhillia involucrata* was collected at an altitude of 1400m on the Roggeveld escarpment, while *P. brevicalyx* and *P. xairuensis* occur in the Overberg region at roughly 200 m above sea level. *Polhillia* species nodulating with *Mesorhizobium* showed more diversity in terms of symbionts. The rhizobial isolate for *P. ignota* had a 99% match with *M. australicum* when blasted to GenBank, which was first isolated from the pasture legume *Biserrula pelecinus* L. in Australia (Nandasena *et al.*, 2009). Another closely related species, *P. obsoleta*, nodulates with a different species of *Mesorhizobium* that was also isolated from *Otholobium virgatum* (Burm.f) C.H.Stirt. (tribe Psoraleeae) with a 100% sequence match on GenBank (Lemaire *et al.*, 2015). *Polhillia connata*, *P. stirtoniana* and *P. curtisiae* all occur in the Overberg and appear to share a potentially novel species of *Mesorhizobium* as rhizobial symbiont. Some rhizobial symbiont patterns appear to show some levels of rhizobial preference as seen in *P. ignota* and *P. obsoleta*, but this is not set in stone, as *Polhillia* symbionts are also shared among different *Polhillia* species as mentioned above. This is particularly important among Overberg species as several narrowly-endemic species occur in close proximity to one another, but never grow in sympatry. Rhizobial symbiont preference was thought to be a factor in maintaining geographical segregation of species, but appears not to be important as the Overberg species *P. brevicalyx* and *P. xairuensis* and also the species *P. connata*, *P. stirtoniana* and *P. curtisiae* share rhizobial symbionts. Future studies should include wider sampling of *Polhillia* rhizobial symbionts, and include other SA Genisteae taxa in order to better infer patterns of rhizobial preference among these genera compared to other groups of Cape legumes.

5. CONCLUSION

This study focussed on *Polhillia* ecology in order to determine what factors potentially influence the distribution of species. We found that differences in the landscape such as slope aspect, gradient and topography are important in maintaining allopatry in *Polhillia* species with overlapping distributions. Soil profiles among species in the Overberg are less variable than between non-Overberg species, but this could be attributed to the small geographic area

in which these six species occur. Importantly though, we found substantial differences in soils between species with overlapping distributions, that along with habitat differences would maintain allopatry for species with overlapping distributions. Closely related species such as *P. curtisiae* and *P. xairuensis* have fairly similar soils, but are geographically distinct and have different habitat preferences, while another closely related species, *P. pallens* grows on substantially different soils. Therefore, a combination of soil and habitat preference may be factors leading to the speciation of closely related species that occur in relatively close proximity. A different pattern is observed between the geographically distinct *P. obsoleta* and *P. groenewaldii* sister species pair in the Breede River valley, where a clear shift in habitat and soil preference has led to the distinction between these species. Rhizobial symbionts were isolated and identified for eight *Polhillia* species, three of which were nodulating with a single lineage of *Rhizobium*, while the remaining five were nodulating with *Mesorhizobium* representing three different lineages. Some rhizobial specificity was seen in *P. obsoleta* and *P. ignota*, but three *Polhillia* species from the Overberg that are not related, but occur in close proximity, shared a rhizobial symbiont. We therefore suggest that topography and soil profile differences play the most important role in maintaining *Polhillia* species allopatry, and where distributions overlap parapatry. Along with geographic isolation, these factors have likely played some role in the speciation of *Polhillia*. These results, however, can only be properly tested through more extensive sampling and the implementation of common greenhouse experiments on both the effects of different soils and rhizobial symbionts on plant growth and persistence.

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CHAPTER 5

CONCLUSIONS

At the onset of this study *Polhillia* C.H.Stirt. comprised of eight species that were considered rare with uncertain species boundaries (Boatwright, 2010; Curtis *et al*., 2013; Stirton, 1986a; van Wyk and Schutte, 1989). Four of the eight species were also listed as Critically Endangered on the South African plant species Red Data List, with the other four species being either Endangered or Vulnerable (Ebrahim *et al*., 2016; Raimondo *et al*., 2009). This resulted in the genus being classified as the third most threatened plant genus in South Africa, only behind *Marasmodes* DC. and *Encephalartos* Lehm. respectively (Ebrahim *et al*., 2016; Magee *et al*., 2017; Raimondo *et al*., 2009). Several of the *Polhillia* species had been previously circumscribed in other genera before being amalgamated into *Polhillia* (Stirton, 1986b, 1986a, 1984; van Wyk, 1992; van Wyk and Schutte, 1989). One of those species was the unique species, *P. involucrata* (Thunb.) B.-E.van Wyk & A.L.Schutte that has pink flowers and dehiscent fruit in a genus otherwise comprised of species with yellow flowers and indehiscent fruit (du Preez *et al*., 2019 – Chapter 2 and 3). This had brought the monophyly of the genus into question and required molecular phylogenetic analysis to confirm the position of this anomalous species. The genus has also never been revised since it was described (Stirton, 1986a), and at least one putative new taxon was noted by Stirton (*pers. comm*.).

Other than the taxonomic questions, very little was also known about *Polhillia* ecology and specifically the edaphic conditions in which these species occur. All *Polhillia* species are confined to Renosterveld vegetation in the Greater Cape Floristic Region (GCFR), with the majority of species residing in the Overberg region of the Western Cape Province (Manning and Goldblatt, 2012; Snijman, 2013). This region is well known for its extensive wheat and canola fields, but less so for the small Renosterveld fragments that persist among the ever expanding agriculture (Cowling *et al*., 1986; Curtis *et al*., 2013; Kemper *et al*., 1999). The soils in this region are fertile for agriculture, but little is known about how soils vary across the landscape and whether soil parameter variation influences the distribution of *Polhillia* species (Cowling *et al*., 1986; Cowling and Holmes, 1992; Richards *et al*., 1997; Thwaites

and Cowling, 1988). Soil parameters are known to influence the distribution of plants across the landscape with Dludlu *et al*. (2018a) showing how such factors influence legumes on the Cape Peninsula. Little was known about which soil parameters potentially influence the distribution of *Polhillia* species in the landscape. As with most legumes, *Polhillia* species form symbiotic relationships with nitrogen-fixing bacteria in the soil called rhizobia (Gualtieri and Bisseling, 2000; Lemaire *et al*., 2015; Sprent, 2007). Rhizobial symbionts of *Polhillia* species have never been thoroughly investigated. Investigating the rhizobial symbionts presented an opportunity to determine whether *Polhillia* species share symbionts or have their own specific symbiont. This investigation also allowed us to sample in areas that have previously not been sampled for rhizobia as most GCFR rhizobia studies have been focused in Fynbos vegetation and on tribes such as Crotalarieae and Psoraleeae (Dludlu *et al*., 2018b, 2018c; Lemaire *et al*., 2015).

In Chapter 2, we tested the monophyly of the genus, and found *Polhillia* to be a monophyletic genus sister to *Argyrolobium* Eckl. & Zeyh. Furthermore, we found that *Polhillia involucrata* strongly resolved as sister to the rest of the genus in our molecular phylogenetic analyses. Based on this, and unique morphological characters (flowers pink or white and pods dehiscent, *versus* flowers yellow and pods indehiscent in other species), we circumscribed *Polhillia involucrata* into a new monotypic subgenus *Roseopolhillia*.

In Chapter 3 we conducted a multidisciplinary systematic revision of the genus to produce a monograph in which we recognise 11 species in the genus *Polhillia*. We reduced *P. canescens* C.H.Stirt. to synonymy under *P. connata* (Harv.) C.H.Stirt. based on a lack of traits to distinguish between the species and attribute differences seen between type specimens to seasonal variation. We also recognized and described four new species: *Polhillia fortunata* B.du Preez, *Polhillia groenewaldii* B.du Preez, *Polhillia stirtoniana* B.du Preez and *Polhillia xairuensis* B.du Preez. The two latter species were split from *Polhillia pallens* C.H.Stirt *s.l.*, while the two former species were both collected for the first time during our extensive fieldwork in 2018. Through our extensive fieldwork and revised species delimitation, we were able to conduct in-depth re-assessments of the conservation status of these threatened species according to the IUCN categories and criteria (IUCN, 2012). Of the 11 species now recognized, only two are listed as Critically Endangered (CR), five as Endangered (EN), three as Vulnerable (VU) and one species as Near-Threatened (NT). This is particularly important as our findings resulted in a status downgrading from CR to EN for

P. curtisiae and *P. ignota*. This is particularly significant for *P. ignota* that we rediscovered back in 2016 after it was considered to be extinct for almost 100 years. The status of *P. connata* was downgraded from CR to VU following the synonymy of *P. canescens*. This chapter serves to showcase the many benefits of conducting a largely field-based study, as species circumscriptions and conservation assessments have greatly benefited from this.

In chapter 4 we investigated habitat characters, soil parameters and rhizobial symbionts of *Polhillia* species in order to determine whether any of them help maintain species allopatry, even when species distributions overlap. We found that habitat variation and certain soil parameters could be responsible for maintaining species allopatry within the Overberg region. Habitat conditions such as landscape topography (hillslope *versus* gully), slope aspect and gradient were found to be important factors in relation to where different species grow. For example, *P. curtisiae* and *P. stirtoniana* have an overlapping distribution, but the former is restricted to mostly north facing hillslopes, while the latter occurs on south facing slopes and in steep gullies. Soils of *P. pallens* and *P stirtoniana* were found to have significantly higher concentrations of potentially toxic metals than other species in the region, which could act as a barrier to other species growing in similar soils. Soils between non-Overberg species are more distinct between species than Overberg species, probably due to their geographic distinctiveness. *Polhillia fortunata* was found to grow in soils with significantly more silt and Na than any other species, while *P. ignota* soils were deficient in cations and metals, while being high in sand and S concentrations. Although rhizobial symbionts do not appear to have as great an influence on the distribution of the various *Polhillia* species, it was interesting to find both *Rhizobium* and *Mesorhizobium* symbionts associated with *Polhillia*. We found that two Overberg species, *P. brevicalyx* and *P. xairuensis*; share a *Rhizobium* symbiont with *P. involucrata* that grows on the Roggeveld escarpment under very different edaphic conditions in terms of altitude and soil composition.

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